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HIV Genome-Wide Protein Associations: a Review of 30 Years of Research

Guangdi Li,[a,b] Erik De Clercq[b]

Department of Metabolism and Endocrinology, Metabolic Syndrome Research Center, Key Laboratory of Diabetes Immunology, Ministry of Education, National Clinical Research Center for Metabolic Diseases, The Second Xiangya Hospital, Central South University, Changsha, Hunan, China[a]; KU Leuven-University of Leuven, Rega Institute for Medical Research, Department of Microbiology and Immunology, Leuven, Belgium[b]

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Address correspondence to Guangdi Li, liguangdi.research@gmail.com, or Erik De Clercq, erik.declercq@kuleuven.be.

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SUMMARY

The HIV genome encodes a small number of viral proteins (i.e., 16), invariably establishing cooperative associations among HIV proteins and between HIV and host proteins, to invade host cells and hijack their internal machineries. As a known example, the HIV envelope glycoprotein GP120 is closely associated with GP41 for viral entry. From a genome-wide perspective, a hypothesis can be worked out to determine whether 16 HIV proteins could develop 120 possible pairwise associations either by physical interactions or by functional associations mediated via HIV or host molecules. Here, we present the first systematic review of experimental evidence on HIV genome-wide protein associations using a large body of publications accumulated over the past 3 decades. Of 120 possible pairwise associations between 16 HIV proteins, at least 34 physical interactions and 17 functional associations have been identified. To achieve efficient viral replication and infection, HIV protein associations play essential roles (e.g., cleavage, inhibition, and activation) during the HIV life cycle. In either a dispensable or an indispensable manner, each HIV protein collaborates with another viral protein to accomplish specific activities that precisely take place at the proper stages of the HIV life cycle. In addition, HIV genome-wide protein associations have an impact on anti-HIV inhibitors due to the extensive cross talk between drug-inhibited proteins and other HIV proteins. Overall, this study presents for the first time a comprehensive overview of HIV genome-wide protein associations, highlighting meticulous collaborations between all viral proteins during the HIV life cycle.

INTRODUCTION

The genome of human immunodeficiency virus (HIV) encodes 16 viral proteins playing essential roles during the HIV life cycle (Fig. 1). Three major genes, *gag*, *pol*, and *env*, code for structural proteins (matrix, capsid, nucleocapsid, and p6), viral enzymes (protease, reverse transcriptase [RT], and integrase), and envelope proteins (GP120 and GP41) (1, 2) (see Text S1 in the supplemental material). The remaining genes code for regulatory proteins (Tat and Rev) and accessory proteins (Vif, Vpu/Vpx, Vpr, and Nef) (3). Vpu is found exclusively in HIV type 1 (HIV-1), whereas Vpx is carried by HIV-2.

Although HIV genomes code for only 16 viral proteins (Fig. 2), a great number of physical interactions between pairs of HIV pro-

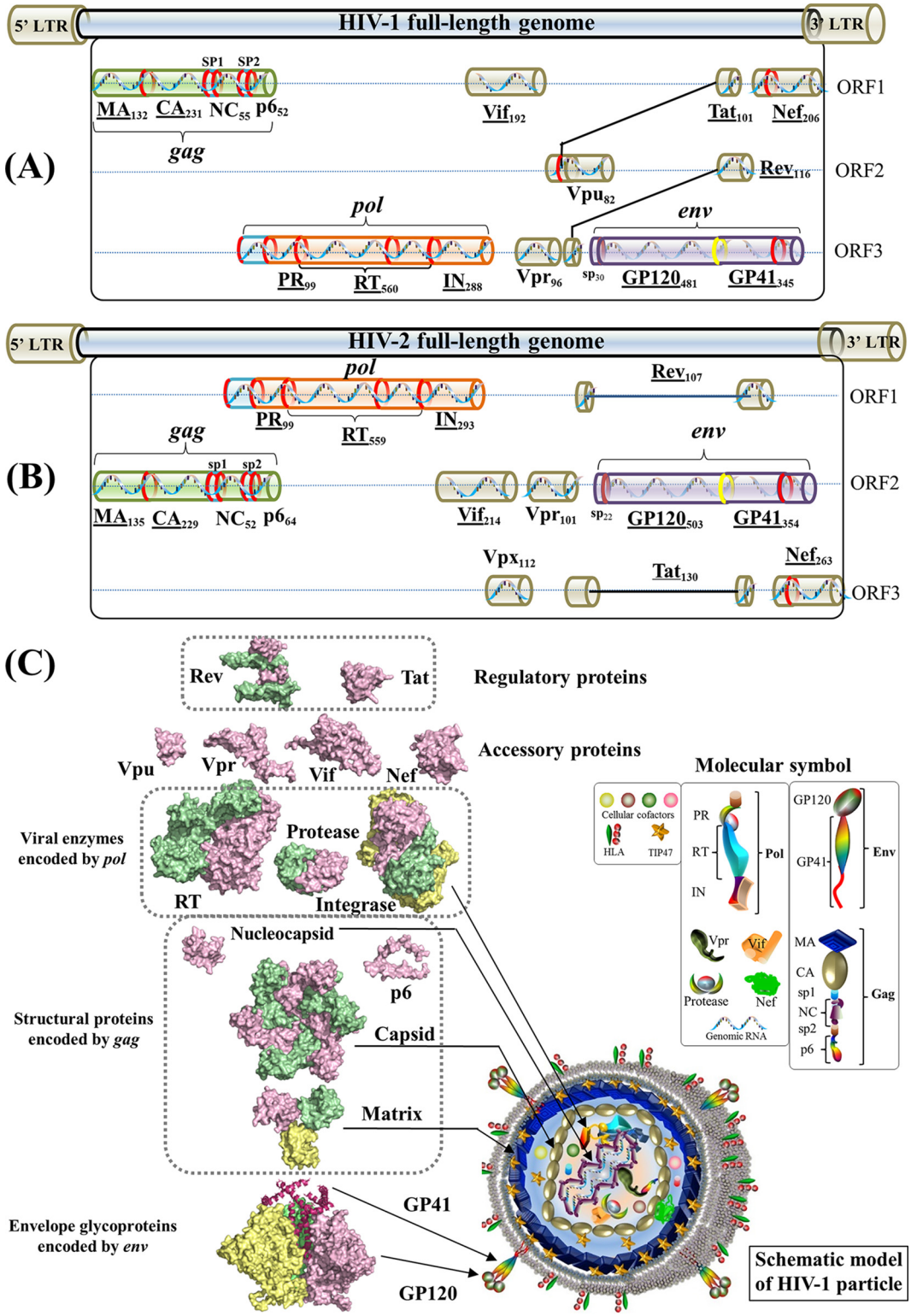


FIG 1 Gene maps and protein structures of HIV-1 and HIV-2. (A) Schematic model of the HIV-1 full-length genome (reference strain HXB2). HIV protein names and amino acid lengths are shown beneath the colored protein regions in three open reading frames (ORFs). Eleven multimeric proteins have underlined names. In the gene map, red rings mark the locations where viral protease cleaves during viral maturation. In the *env* gene, a yellow ring shows the cleavage position of human proteases (e.g., furin) (594). The 5′ and 3′ long terminal regions (LTRs) are also indicated in the full-length genome. (Adapted from reference 44.) (B) Schematic model of the HIV-2 full-length genome (reference strain SIVmac239). (Adapted from reference 44.) (C) Surface representations of HIV-1 protein structures and schematic view of the HIV-1 particle. Surface representations of 15 HIV-1 protein structures are clustered according to their functional roles. HIV-1 monomeric proteins are shown in pink, and different subunits of multimeric proteins are distinguished with different colors (green, yellow, and red). HIV-1 protein structures are scaled precisely for a direct and intuitive comparison. At the bottom right, a schematic model of a mature viral particle is displayed, and the key shows protein annotations. Proteins in the schematic view are shown for illustration purposes; their structures and sizes here are not necessarily identical to the real protein structures and sizes. Additional information about HIV genomic reference sequences and natural polymorphisms is available online (see http://www.virusface.com/). (Adapted from reference 44.)

teins, so-called HIV pairwise protein interactions, provide essential mechanisms for HIV to achieve efficient viral replication at different stages of the HIV life cycle (4). For instance, the HIV-1 envelope glycoprotein GP120 physically interacts with GP41 during viral entry (5). In addition to HIV pairwise protein interactions, HIV-host protein interactions are known to play essential roles for HIV to hijack human cellular systems (6–11). Because of this, functional associations between HIV proteins can be mediated via host molecules (e.g., CD4). Taking the functional association of Vpu-CD4-Env as an example, the binding of Vpu to CD4 facilitates the proper assembly of Env into HIV-1 particles, because Vpu interacts with CD4 to trigger the rapid degradation of newly synthesized CD4, thereby preventing the aggregation of CD4-Env structural complexes in the endoplasmic reticulum (ER) (12–19). Overall, physical interactions and functional associations between 16 HIV proteins delineate a global perspective of HIV genome-wide associations that play essential roles during the HIV life cycle.

To our knowledge, a systematic review that provides a genome-wide perspective on HIV pairwise protein associations is still lacking in spite of many studies focusing on individual protein associations. In theory, 16 HIV proteins would generate 120 pairwise protein associations, but some associations might be absent during the HIV life cycle. To disclose the mystery of HIV protein associations from a genome-wide perspective, we thus performed the first systematic review to establish experimental evidence for HIV pairwise protein associations and their functional activities at major stages of the HIV life cycle: viral entry (20–22), reverse transcription (23), viral integration (24–26), viral transcription and translation (27–29), viral assembly and budding (2, 30), and viral maturation (2, 30, 31) (Fig. 3).

Based on a large body of publications accumulated from 1985 to 2015, our review is focused on the following three aspects. (i) What molecular experiments were used to report HIV protein associations? (ii) Where and when do HIV protein associations achieve their functional activities during the HIV life cycle? (iii) Which viral protein domains are responsible for protein interactions at the molecular level? Clinical relevance and therapeutic implications of HIV genome-wide protein associations are discussed from two aspects: novel mechanisms of HIV drug resistance and HIV-derived peptide inhibitors. The former provides new insights into why HIV-infected patients could fail highly active antiretroviral therapies (HAARTs) in the absence of drug-resistant mutations. The latter may shed light on the development of anti-HIV agents.

Our review begins with the procedure for literature selection. Thereafter, physical interactions and functional associations between HIV proteins are described, depending on their activities at major stages of the HIV life cycle. For each physical interaction or functional association, its biological activities and interaction domains are summarized. For a better understanding of HIV protein associations, we visualize protein interactions during the HIV life cycle, discuss their clinical relevance and therapeutic implications, and establish an online platform to update the information on HIV genome-wide protein associations (http://www.virusface.com/). Newly created structural movies have been shared online to highlight protein interaction domains. Challenges and future perspectives are discussed at the end of this review.

LITERATURE SELECTION

This section describes the procedure for our literature selection (Fig. 4). We performed an electronic literature search by querying English articles from three sources (PubMed, Google Scholar, and Cochrane Library) plus reference lists of retrieved articles published from January 1985 until December 2015. Moreover, we extracted literature from the HIV-1 Human Interaction Database (HHID) to collect information about HIV-host protein interactions (32). This extraction allows the identification of any cellular protein that physically interacts with two HIV proteins. For instance, the Vif-APOBEC3G-integrase association exists because the cellular protein APOBEC3G physically interacts with viral Vif and integrase during the HIV life cycle (33–43). In summary, three major steps were carried out by selecting studies that reported protein associations involving 16 HIV proteins (matrix, capsid, nucleocapsid, p6, protease, RT, integrase, Vif, Vpr, Vpu, Vpx, Tat, Rev, GP120, GP41, and Nef) and/or 2 precursor proteins (Gag and Env).

Step 1 was an electronic search. We searched English articles through four sources (PubMed, Google Scholar, the Cochrane Library, and the HHID), given the publication period from January 1985 until December 2015. Search terms covered all pairwise associations between 18 HIV (precursor) proteins, resulting in 153 keyword patterns (e.g., "HIV matrix capsid," "HIV matrix nucleocapsid," and "HIV matrix p6"). Article titles and abstracts from these databases were scrutinized, except for Google Scholar, by which we examined only the top 100 publications for each keyword pattern due to a great mass of results found. We also queried review articles about the functions and interactions of individual HIV proteins. Thereafter, we gathered publications that met three selection criteria (see below).

Step 2 included manual reference checks of extracted publications. To search the literature on HIV pairwise protein associations, we manually checked the reference list of each publication extracted by using step 1. Publications that met the selection criteria (see below) were selected.

Step 3 included citation tracking in Google Scholar. Using Google Scholar, we manually checked publications that cited those articles retrieved by using step 2. Thereafter, newly identified publications were collected for the next search round through step 2. The search process was terminated if new publications could not be found.

Articles were selected for our review if they met any of the following selection criteria:

1. Protein-protein interaction (PPI) experiments. We retrieved English articles that demonstrated HIV PPIs or their biological functions using *in vitro* or *in vivo* experiments (e.g., coimmunoprecipitation assays, glutathione *S*-transferase [GST] pulldown assays, two-hybrid assays, enzyme-linked immunosorbent assays [ELISAs], Western blot assays, dot blot assays, electron microscopy analysis, X-ray crystallography, nuclear magnetic resonance [NMR] spectroscopy, and surface plasmon resonance analysis). Articles that reported the absence of a physical interaction between two HIV proteins were also selected. However, we discarded prediction-based studies that only hypothesized PPIs without any experimental proof of physical interactions.
2. Statement of PPI functions. We retrieved articles that clearly expressed the functional relationship between two

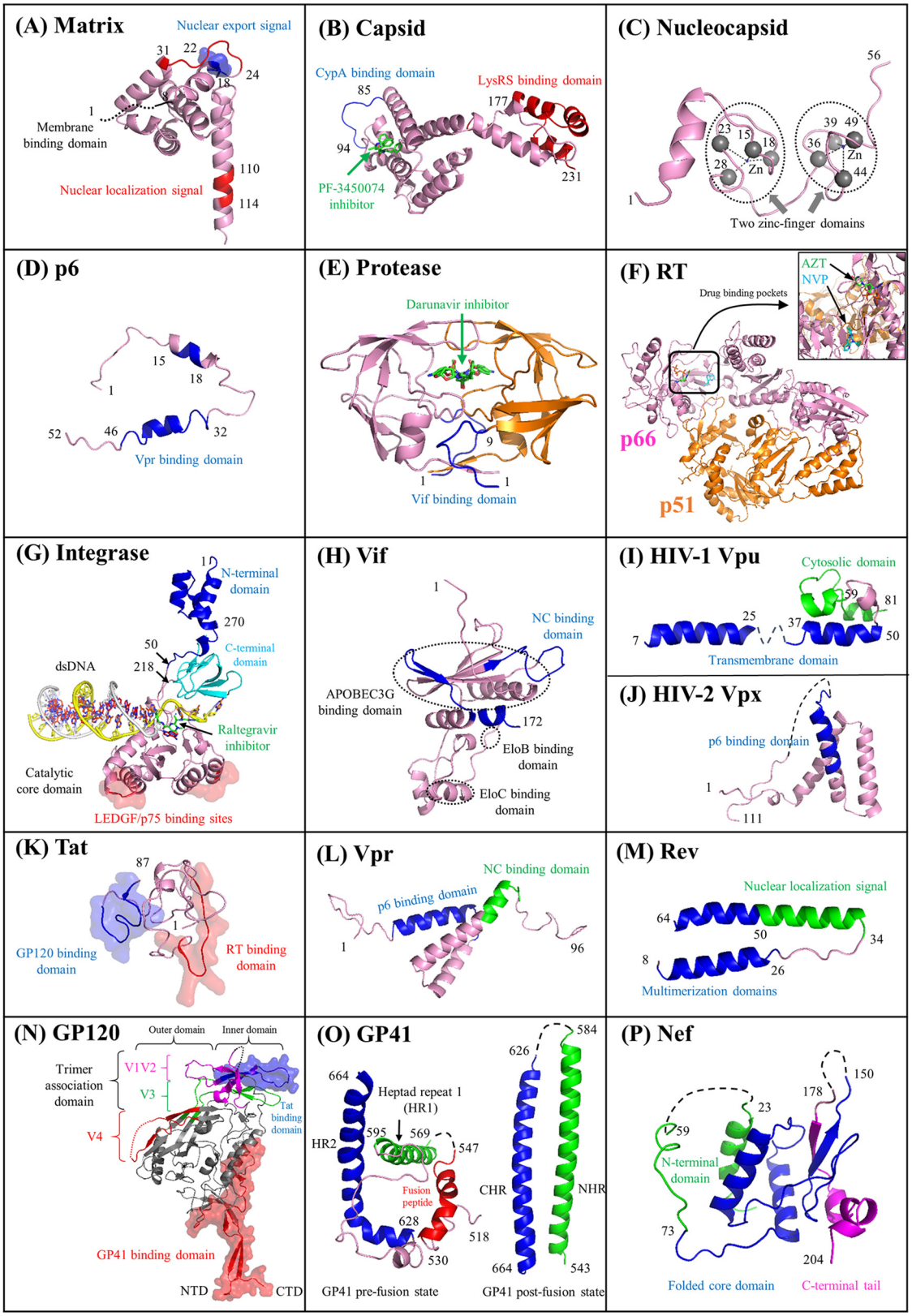


FIG 2 Functional domains of 16 HIV proteins. Cartoon representations of 16 HIV proteins (matrix, capsid, nucleocapsid, p6, protease, RT, integrase, Vif, Vpu, Vpx, Tat, Vpr, Rev, GP120, GP41, and Nef) are visualized. For each panel, protein domains involved with HIV pairwise protein interactions are marked accordingly. Surface representations indicate protein interaction interfaces. Distinct functional domains are annotated in different colors, such as the N-terminal heptad repeat (NHR) and the C-terminal heptad repeat (CHR) of GP41 in panel O. The V1 to V4 flexible loop regions of GP120 (see details in reference 50) are

HIV proteins with terms such as "interact," "bind," "associate," "packaging," "incorporate," "inhibit," "activate," "promote," "cleave," "enhance," "degradation," "upregulate," and/or "downregulate."

3. Protein interaction domains. We retrieved articles that reported interaction domains of HIV pairwise protein interactions. Amino acid positions were indexed by using HIV reference strains (HIV-1 reference strain HXB2 and HIV-2 reference strain BEN) (44).

As for HIV-host protein interactions, we also performed a similar literature search. If different studies suggested incomparable results on HIV protein interactions, they were presented for discussion. Different results for interaction positions are listed separately based on the original publications (Table 1). Biological experiments used for the identification of HIV protein interactions are summarized in Table 2. HIV functional associations mediated by a host protein or a viral factor are summarized in Table 3.

VIRAL ENTRY

During viral entry, HIV particles penetrate host cells and initiate cell infection (Fig. 3). Host cells (e.g., T-helper cells, monocytes, macrophages, and dendritic cells), which express the CD4 (cluster of differentiation 4) glycoprotein on the cell surface, are the primary targets of HIV Env spikes, structural complexes formed by HIV GP120 and GP41 (Fig. 5). HIV entry pathways, entry inhibitors, and HIV-associated human proteins have been reviewed elsewhere (20, 21, 45–47). Here, we focus on HIV pairwise protein interactions during viral entry.

GP120-GP41 Interaction

On the surface of HIV particles, GP120 physically interacts with GP41 to construct trimeric Env spikes via noncovalent interactions (48–58). During viral entry, HIV Env spikes undergo dynamic structural rearrangements to invade host cells (51, 52, 59) (Fig. 5). When GP120 binds to the cellular receptor CD4 on the host cell, this binding induces an outward domain shift of GP120 subunits to disrupt noncovalent interactions between GP120 and GP41 and to expose coreceptor-binding sites (60). Thereafter, GP41 helices at the core of Env spikes serve as anchors by which the rest of Env can be reorganized into open structural conformations for viral entry (48). Specifically, prefusion GP41 wraps its hydrophobic core around the extended N-terminal domain (NTD) and the C-terminal domain (CTD) of GP120 to construct a GP41-tryptophan clasp (50, 56). In comparisons of the prefusion and postfusion conformations of GP41 (Fig. 2), it has been shown that the spike rearrangements open the GP41-tryptophan clasp to expel GP120 termini, thereby constructing a fusion pore for viral entry (50, 61). Although it remains debated (22), the construction of a fusion pore may require 1 to 7 Env spikes for entry stoichiometry, with most HIV strains depending on 2 to 3 Env spikes (62).

Extensive studies have elaborated interaction domains of the GP120-GP41 interaction (48–58). It is generally agreed that the inner domain and the N- and C-terminal domains of GP120 maintain noncovalent interactions with the heptad repeat 1 and disulfide-bonded domains of GP41 (Fig. 2). Particularly, the GP120 inner domain can modulate the GP120-GP41 interaction and CD4 binding (51), while GP120 terminal regions mainly interact with the disulfide-bonded region of GP41 (55, 63–65). Amino acid substitutions (e.g., W596A and W610A) within the GP41 disulfide-bonded region disrupt the GP120-GP41 interaction (66). In addition, broadly neutralizing antibodies (e.g., 3BC315) have been identified to interrupt the GP120-GP41 interaction, but the dynamic nature of the Env trimers may influence the exposure of antibody epitopes (67, 68). Table 1 summarizes findings from a recent X-ray crystallographic study that unveils the interaction positions of GP120 and GP41 in the atomic structure of HIV-1 Env (50). Last but not least, the GP120-GP41 interaction exerts an impact on drug resistance to HIV entry inhibitors, a novel mechanism of HIV drug resistance which is described in detail below.

GP41^{Env}-Matrix^{Gag} Interaction

GP41^{Env} has been detected to physically interact with HIV-1 matrix in Gag precursors (matrix^{Gag}) (69–76) (Table 2). The cytoplasmic tail of GP41 (GP41CT) not only enhances Env packaging during viral budding (77, 78) but also drives the rearrangements of Env prebundle structures during viral entry (79). The GP41^{Env}-matrix^{Gag} interaction undertakes multiple activities. (i) HIV-1 entry is suppressed by the interaction between the GP41CT and unprocessed Gag in immature HIV-1 particles (80). However, this suppression is dismissed when HIV-1 protease cleaves Gag and GagPol precursors, a maturation process that transforms immature HIV-1 particles into mature HIV-1 particles (80). (ii) Differential localization of Env trimers on the viral surface depends on the GP41^{Env}-matrix^{Gag} interaction, because the proteolysis of Gag rearranges the inner protein lattice to alter the clustering of Env for viral entry (81). (iii) Matrix^{Gag} prevents access of the GP41CT to biotinylation (82).

Regarding the interaction domains, the basic and C-terminal domains of HIV-1 matrix^{Gag} physically interact with the GP41CT (72, 75, 83). Mutagenesis analyses suggest that the matrix substitution L49D destabilizes the GP120-GP41 interaction, but this impairment can be rescued by a Y710S substitution at the GP41CT (84). The last 13 to 43 amino acid positions in the GP41CT are critical for the GP41^{Env}-matrix^{Gag} interaction (73). In addition, GP41CT mutations may confer resistance to HIV protease inhibitors (PIs) (85), a mechanism which is described in detail below.

mapped in panel N. Five small molecules shown in green elucidate protein inhibitors such as the capsid inhibitor PF-3450074 (503) (B), the protease inhibitor darunavir (E), the nucleoside analogue reverse transcriptase inhibitor zidovudine (AZT) (F), the nonnucleoside analogue reverse transcriptase inhibitor nevirapine (NVP) (F), and the integrase inhibitor raltegravir (G). HIV-1 protein domains that interact with cellular proteins are mapped, such as AIP1 (376) (C); LEDGF/p75 (168) (G); and APOBEC3G, EloB, and EloC (595) (H). For some HIV-1 multimeric proteins (matrix, capsid, integrase, Rev, GP120, and GP41), only a subunit is demonstrated, and their multimeric structures are shown in Fig. 1. Text S1 in the supplemental material describes major functions of 16 HIV proteins. Except for HIV-2 Vpx, PDB data for the other 15 proteins were obtained for HIV-1. A list of PDB accession numbers used for our structural visualization is available in Table S1 in the supplemental material. The integrase structure of prototype foamy virus is used for visualization purposes, because the full-length structure of HIV integrase is lacking. HIV structural movies and teaching slides are available at our online platform (see http://www.virusface.com/).

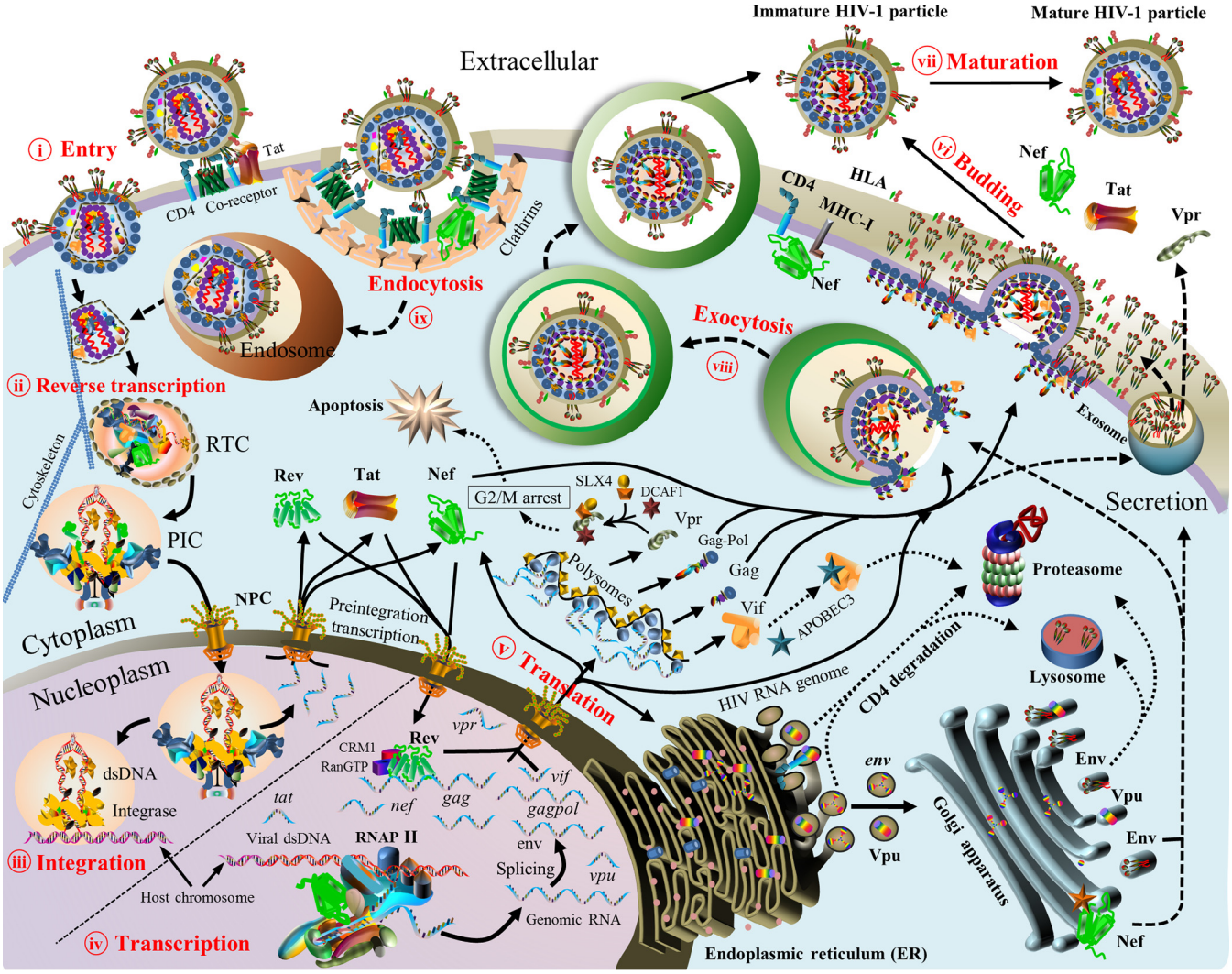
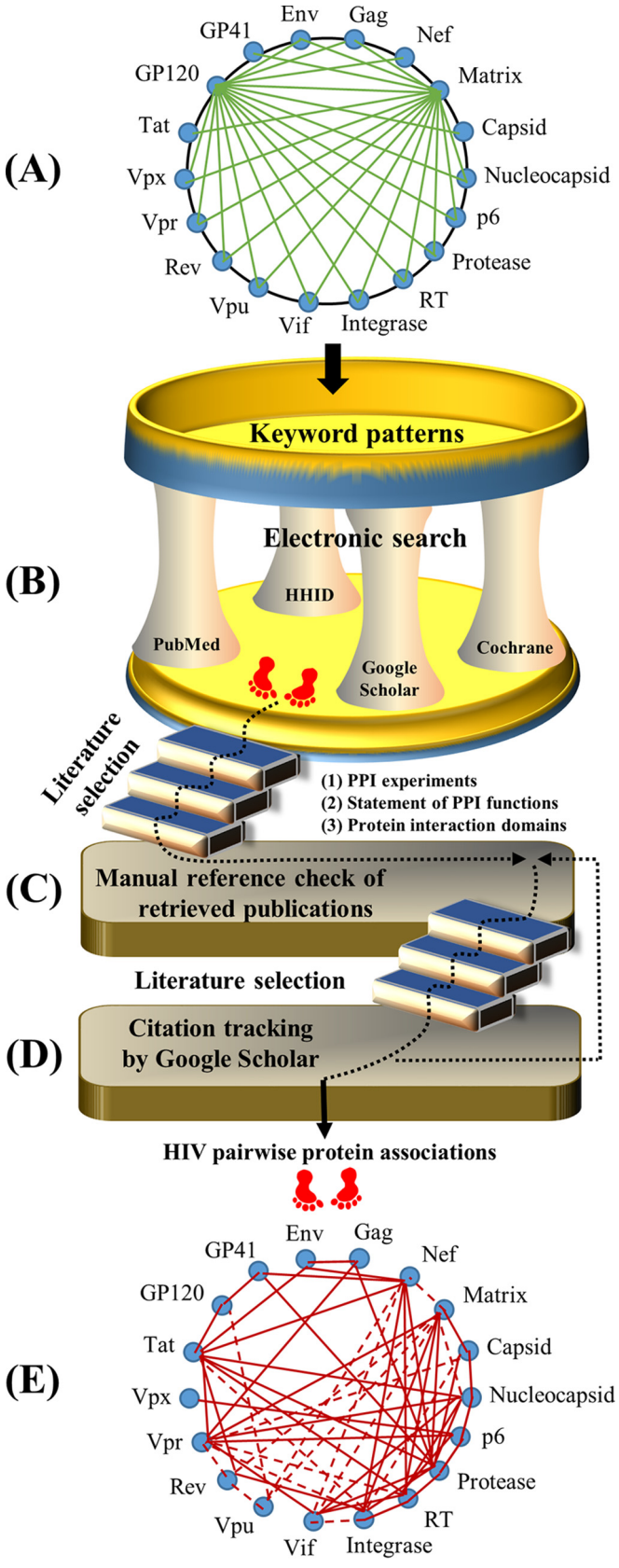


FIG 3 Overview of the HIV-1 life cycle. Nine stages are described. (i) Viral entry (viral fusion). Mature HIV virions target host cells through direct binding to the cellular receptor CD4 and chemokine coreceptors (e.g., CCR5 and CXCR4) (20, 21). (ii) Reverse transcription. HIV reverse transcriptase in the reverse transcriptase complex (RTC) produces double-stranded DNA (dsDNA) from single-stranded RNA (23). (iii) Viral integration. The HIV preintegration complex (PIC) transports viral dsDNA into the nucleus by entering the nuclear pore complex (NPC). During preintegration transcription, three viral proteins (Rev, Tat, and Nef) are synthesized from unintegrated dsDNA (25). In the presence of cellular cofactors (e.g., LEDGF/p75), the PIC targets host chromosomal regions with high transcriptional activity, where viral dsDNA is integrated into host chromosomes (24). (iv) Viral transcription. Viral proteins (Tat and Nef) hijack cellular transcription machineries to activate viral mRNA synthesis from integrated viral dsDNA (29). HIV Rev recruits CRM1, RanGTP, and other host proteins to export viral mRNAs into the cytoplasm (213). (v) Viral translation. Viral mRNAs are translated into (precursor) proteins in the cellular compartments. Viral mRNAs of Gag, GagPol, and most accessory proteins are translated in cytosolic polysomes, except for Env and Vpu (30). In the cytoplasm, mature Vpr interacts with host proteins (DCAF1 and SLX4) to induce G_2/M cell cycle arrest (236, 596), Vif activates the degradation of APOBEC3 proteins (34, 233), and Nef plays multiple roles in different cellular compartments based on HIV-host protein interactions (597). In the ER, HIV-1 Vpu mRNA is translated, and mature Vpu retains newly synthesized CD4 (19). The dislocated CD4 thereafter undergoes lysosomal and/or proteasomal degradation (19, 451). Tetherin is also targeted by Vpu in the ER, and it is subsequently delivered to the lysosomal and/or proteasomal degradation pathways (459, 461). In the ER, modifications of Env such as signal peptide cleavage, folding, trimerization, and glycosylation occur (598). In the Golgi apparatus, cellular proteases (e.g., furin) cleave Env glycoproteins into GP120 and GP41, which are subsequently assembled into Env spikes via noncovalent interactions (599). Most Env proteins retained in the ER or Golgi apparatus are degraded, and only a small proportion reaches the cell membrane (600). Env, Vpr, Tat, and Nef travel to the cell membrane via secretory pathways (30, 601–603). (vi) Viral assembly and budding. Nascent HIV virions are assembled with two genomic mRNAs, viral proteins (Gag, GagPol, Env, Vif, Vpr, and Nef), and cellular cofactors (e.g., actin, $tRNA^{Lys3}$, and TIP47) (2, 30, 277). Nascent HIV particles pinch off from the cellular membrane to infect other host cells (30). HIV-1 Nef induces CD4 degradation to prevent the Env-CD4 interaction on the extracellular membrane (597, 604). (vii) Virus maturation. HIV protease cleaves Gag and GagPol precursors into structural proteins (matrix, capsid, nucleocapsid, and p6) and viral enzymes (protease, RT, and integrase), thus transforming immature virions into mature virions for new infections (2, 30, 31). (viii) Virus exocytosis. As an alternative route of viral budding, nascent HIV virions are released by using exocytosis pathways (605). (ix) Virus endocytosis. As an alternative route of viral entry, mature HIV virions enter host cells through endocytic pathways (46). Note that protein shapes do not represent the exact protein structures, nor are the protein sizes to scale. MHC-I, major histocompatibility complex class I.



GP120-Tat Interaction

HIV-1 Tat can physically interact with GP120, an interaction detected by isothermal titration calorimetry, pulldown assays, ELISAs, electron cryomicroscopy, and surface plasmon resonance analyses (86–89). Although Tat is dispensable for viral entry, the binding of Tat to GP120 contributes to efficient viral entry (86), and Tat-mediated viral entry promotes the infection of monocyte-derived dendritic cells (88). After HIV-infected cells release Tat to the extracellular space (86, 90), the GP120-Tat interaction undertakes multiple activities. (i) Extracellular Tat binds to Env spikes, a process which blocks the recognition of anti-Env antibodies, allowing HIV to escape from Env neutralization (88). Furthermore, extracellular Tat interacts with chemokine receptors (e.g., CCR2 and CCR3) to recruit chemokine receptor-expressing monocytes and macrophages toward HIV-infected cells (91). (ii) The released Tat physically interacts with GP120 on the extracellular membrane of uninfected cells (86). By doing so, Tat induces the aggregation of Env trimers to adapt conformational changes for viral entry (92). (iii) The GP120-Tat interaction might affect the switch of viral coreceptor tropism, because after its interaction with Tat, GP120 of an X4-tropic virus efficiently interacts with CXCR4 and CCR5 (92). Although the GP120-Tat interaction has an impact on viral entry, it does not affect Tat-mediated transactivation (86).

Regarding the GP120-Tat interaction domains, molecular docking analyses suggest that the CD4-binding site and the V3 loop of GP120 may interact with the cysteine-rich domain of Tat (87, 88). Other studies have also proposed the binding of the V1/V2 loop of GP120 to the second exon of Tat (86, 89) (Fig. 2). Further analyses are still needed to examine whether this disagreement is due to dynamic protein interactions on the extracellular membrane or due to different experimental settings.

REVERSE TRANSCRIPTION

During HIV reverse transcription, RT produces a double-stranded DNA (dsDNA) genome from a single-stranded RNA genome (23) (Fig. 3). After viral entry, a series of events take place in the viral core for the establishment of the reverse transcriptase complex (RTC) (Fig. 6). Although its exact composition is still a topic of debate, the HIV-1 RTC may consist of RT, protease, integrase, matrix, capsid, nucleocapsid, Vif, Tat, Nef, Vpr, and host proteins

FIG 4 Work flow for our literature selection. (A) HIV keyword patterns. Sixteen HIV proteins and two precursor proteins (Gag and Env) are shown surrounding a circle. Pairwise HIV proteins are annotated to exhibit keyword patterns (e.g., matrix-capsid, matrix-nucleocapsid, and matrix-p6), exemplified by green links bridging one protein to the other proteins. (B) Electronic search. Given the 18 HIV (precursor) proteins, there are 153 pairwise keyword patterns (i.e., combinations of two proteins). Each pairwise keyword pattern was queried by using four sources (PubMed, Google Scholar, the Cochrane Library, and the HIV-1 Human Interaction Database [HHID]). Publications were thereafter retrieved based on three selection criteria: PPI experiments, statement of PPI functions, and protein interaction domains (see Literature Selection). (C) Reference search. We manually checked the reference lists of articles retrieved as described above for panel B. These articles were also examined by the three selection criteria described above. (D) Citation search. Google Scholar was used to retrieve publications that cited the articles retrieved as described above for panel C. If articles supporting HIV pairwise protein associations were newly identified, we returned back to the step described above for panel C to manually check their reference lists. The searching procedure was terminated when new publications could not be identified. (E) Summary of HIV protein associations. Literature information was summarized, for instance, by networks of HIV pairwise protein associations.

TABLE 1 Summary of HIV pairwise protein interactions[a]

Protein 1-protein 2	Life stage(s)	Major function(s)	Positions in protein 1 (reference[s])	Positions in protein 2 (reference[s])	Reference(s)
GP120-GP41	Entry, budding	Promotes viral entry, promotes viral budding, promotes Env packaging	31–46, 50–54, 70–75, 84–89, 91, 103, 106, 107, 110, 111, 114, 215, 220–224, 226, 244, 246, 489–505 (50)	520–528, 530, 533, 534, 536, 537, 540, 541, 543–546, 569–572, 574, 575, 577–579, 581, 582, 585, 586, 588–592, 593, 596–598, 601–610, 614, 617–619, 622, 623, 628, 629, 631–633, 635, 636, 639, 642, 643, 646, 650, 651, 654, 658, 659, 661–663 (50)	48–58
$GP41^{Env}$-matrix	Entry, budding	Promotes viral entry, promotes Env packaging	712, 713–856 (70, 84); 764–856 (591); 814–844 (73)	12, 30, 34 (76); 18, 20, 22, 32, 33 (83, 592); 6, 29, 31, 62 (74); 49 (84); 62 (72); 84, 99 (75)	69–76, 83, 84, 591, 592
GP120-Tat	Entry	Promotes viral entry	166–171 (89); 157–171 (86)	73–86 (86)	86, 89
RT-integrase	Reverse transcription, integration	Enhances RT activity, inhibits integrase activity	1–242, 387–421 (108)	201–288 (108); 243, 250, 258, 220–270 (102); 213–288 (106)	102, 106, 108
RT-nucleocapsid	Reverse transcription	Enhances RT activity	548–560, p51 domain (113)	13–30, 34–51 (110, 111)	110, 111, 113
RT-Vif	Reverse transcription	Enhances RT activity	?	161–164	131
RT-Tat	Reverse transcription	Enhances RT activity	?	49–57	139
RT-Nef	Reverse transcription	Enhances RT activity	p51 domain	154–172	148
Protease-Tat	Reverse transcription	Protease cleaves Tat to enhance reverse transcription	Protease catalytic site	49–57	152
Integrase-Rev	Integration	Inhibits integrase activity	66–80, 118–128 (173)	12–23, 53–67 (173)	173–175, 180
Integrase-matrix	Integration	Enhances nuclear import of the PIC	50–212	132	181
Matrix-Vpr	Integration	Enhances nuclear import of the PIC	88–132	?	183
Integrase-Nef	Integration	?	?	58–206	149
Tat-Vpr	Transcription	Enhances viral transcription	50–67	?	220
Tat-Rev	Transcription	Proteasomal degradation of Tat	?	35–50	216
Tat-Nef	Transcription	Enhanced viral transcription	?	?	223
Tat-nucleocapsid	Transcription	NC induces Tat degradation	?	?	228
Vif-Vpr	Transcription	Vif mediates Vpr degradation	?	?	232
NC^{Gag}-Vif	Budding	Vif packaging, viral core stability, inhibits PR cleavage	44–55 (301)	75–114 (300)	300, 301, 303
NC^{Gag}-Vpr	Budding	Vpr packaging	13–30, 34–51	70–80	313
$p6^{Gag}$-Vpr	Budding	Vpr packaging	15–18 (329); 32–46 (325); 34–36 (326); 35–47 (330); 41–46 (322)	18–34 (322); 1–71 (327)	322, 325–327, 329, 330
$p6^{Gag}$-Vpx	Budding	Vpx packaging	15–40 (333)	73–89 (333)	314, 332, 333
$GP41^{Env}$-Nef	Budding	Env packaging	712–715	181–210	336
Gag-RT	Budding	RT packaging	183–305 (347)	?	347, 348
Protease-Gag/GagPol	Maturation	Gag and GagPol cleavage	Protease catalytic site	Gag/GagPol cleavage sites	495–497
Protease-Vif	Maturation	Inhibits protease activity	1–9 (516, 517)	30–65 (519); 41–65 (520); 78–98 (516); 81–88, 88–98 (521)	516, 517, 519–521
Protease-RT	Maturation	Protease cleaves RT^{GagPol}, RT promotes protease activity, protease inhibits RT activity	Protease catalytic site	440 | 441	522, 593
Protease-Nef	Maturation	Protease cleaves Nef, Nef inhibits protease activity	Protease catalytic site	57 | 58	528
Protease-GP41CT	Maturation	Protease cleaves GP41CT	Protease catalytic site	714 | 715, 716 | 717	538

[a] A question mark indicates that the corresponding information is not available. A vertical line indicates a protease cleavage site (e.g., 57 | 58 suggests that HIV protease cleaves the substrate protein between amino acid positions 57 and 58). Position indices of GP120 and GP41 are based on the Env protein sequence in the HIV-1 HXB2 reference strain. Except for HIV-2 Vpx, interaction domains are reported for HIV-1.

TABLE 2 Summary of experiments that confirm HIV pairwise protein interactions[a]

Protein interaction	Confirmation of protein interaction by:						Reference(s)
	IP	Pulldown	Two-hybrid assay	Western/dot blotting	X-ray/EM/ NMR/SPR	Others	
GP120-GP41					Yes	Yes	48–58
$GP41^{Env}$-matrix	Yes			Yes		Yes	69–76
GP120-Tat		Yes			Yes	Yes	86–89
RT-integrase	Yes	Yes		Yes	Yes		101–108
RT-nucleocapsid	Yes			Yes		Yes	110–113
RT-Vif		Yes					131
RT-Tat	Yes	Yes	Yes				139
RT-Nef	Yes	Yes				Yes	148, 149
Integrase-Rev	Yes	Yes					173–175
Integrase-matrix	Yes						181
Matrix-Vpr	Yes		Yes				183
Integrase-Nef	Yes	Yes	Yes				149
Tat-Vpr	Yes	Yes					220
Tat-Rev	Yes	Yes	Yes				216
Tat-Nef	Yes	Yes				Yes	223
Tat-nucleocapsid	Yes	Yes	Yes			Yes	228
Vif-Vpr	Yes						232
NC^{Gag}-Vif	Yes	Yes	Yes			Yes	300–303
NC^{Gag}-Vpr			Yes	Yes		Yes	312–314
$p6^{Gag}$-Vpr	Yes	Yes	Yes			Yes	314, 315, 320–330
$p6^{Gag}$-Vpx	Yes	Yes	Yes			Yes	314, 320, 332, 333
$GP41^{Env}$-Nef						Yes	336
Gag-RT	Yes			Yes			347, 348
Protease-Gag/GagPol					Yes	Yes	495–497
Protease-Vif	Yes					Yes	282, 516, 517
Protease-RT	Yes					Yes	522
Protease-Tat						Yes	152
Protease-Nef						Yes	528–532
Protease-GP41CT						Yes	538, 539

[a] IP, co- or radioimmunoprecipitation assay; pulldown, GST pulldown assay; two-hybrid assay, yeast or mammalian two-hybrid assay; Western/dot blotting, (far-)Western blot or dot blot assay; X-ray/EM/NMR/SPR, X-ray crystallography, electron microscopy analysis, NMR spectroscopy analysis, or surface plasmon resonance analysis; others, other cell culture or cell-free experiments (e.g., mass spectrometry) used for the identification of HIV pairwise protein interactions.

(93–95). Notably, HIV capsid and a small subset of phosphorylated matrix are weakly associated with viral DNA (95, 96). During reverse transcription, the RTC produces viral dsDNA with a high content of uracil that protects viral dsDNA from viral autointegration (97). HIV autointegration is a suicidal process in which viral dsDNA is integrated within itself by viral integrase (98).

As of today, it remains a topic of debate as to where and when HIV reverse transcription occurs. Recent evidence favors the hypothesis that reverse transcription takes place in the intact capsid core (96) and is triggered by the presence of massive amounts of deoxyribonucleotides in the cytoplasm (99). Thereafter, the intact capsid core moves toward the nuclear pore, during which the RTC is reconstructed into the preintegration complex (PIC) (Fig. 6). Different aspects of HIV reverse transcription have been reviewed elsewhere, for instance, enzymatic activities of HIV-1 RT (23), the maturation of the RTC (93), strand transfer reactions, and recombinant events (100). Here, we focus on HIV-1 pairwise protein interactions and associations that take place during reverse transcription.

RT-Integrase Interaction

HIV-1 RT has been identified to physically interact with viral integrase by using GST pulldown assays, coimmunoprecipitation assays, dot blot assays, NMR spectroscopy analyses, and surface plasmon resonance analyses (101–108). The binding of integrase to RT does not require multimeric integrase or an integrase with complete enzymatic activity (108). Owing to the integrase-RT interaction, HIV-1 integrase plays an important role in the initiation of reverse transcription (104). Although viral integrase exerts no influence on steps at or before template-primer annealing, it acts at the early stages of reverse transcription by stimulating the initiation and elongation of viral DNA synthesis (109). Of interest, the RT-integrase interaction exerts an impact on drug resistance to HIV RT inhibitors (RTIs) and integrase inhibitors (INIs), a novel drug resistance mechanism that is described below.

Regarding the interaction domains, the C-terminal domain of integrase may interact with RT (102, 104, 106, 107). Mutagenesis analyses also suggest that integrase mutations at the catalytic core domain (e.g., C130S) and the C-terminal domain (e.g., W243E, V250E, and K258A) could severely diminish the RT-integrase interaction, thereby impairing reverse transcription (102, 106). Moreover, the finger-palm domain (positions 1 to 242) and the C terminus of the connection subdomain (positions 387 to 421) in RT may interact with integrase (108). However, the exact interaction positions remain unclear.

RT-Nucleocapsid Interaction

HIV-1 RT physically interacts with nucleocapsid according to far-Western blot, chemical cross-linking, and coimmunoprecipita-

TABLE 3 Summary of HIV pairwise protein associations

Protein association[a]	Life stage	Major function(s)	References
RT-tRNA^{Lys3}-Vpr	Reverse transcription	Vpr interacts with tRNA^{Lys3} to inhibit initiation of reverse transcription	155–159
Integrase-dsDNA-Vpr	Integration	Vpr promotes binding of integrase to dsDNA	24, 187, 189
Integrase-dsDNA-nucleocapsid	Integration	Nucleocapsid stabilizes integrase binding to DNA and promotes the integrase strand transfer reaction	191–194
Integrase-TNPO3/CypA-Capsid	Integration	Integrase and capsid interact with TNPO3/CypA to facilitate PIC nuclear import and viral integration	195–197, 203, 204
Rev-CRM1-matrix^{Gag}	Translation	Rev and matrix^{Gag} recruit CRM1 and cellular cofactors for nuclear export of viral mRNA	239–242
Rev-CG1-Vpr	Translation	Rev and Vpr bind to CG1 for mRNA nuclear export	246–248
Tat-p300/SWI/SNF-integrase	Transcription	p300/SWI/SNF promotes Tat-mediated viral transcription and integrase-mediated viral integration	251–256, 266–272
Matrix^{Gag}-RNA-NC^{Gag}	Budding	Viral genomic RNA binds to matrix^{Gag} and NC^{Gag} for viral RNA incorporation	350–360
Capsid^{Gag}-LysRS-Vpr	Budding	LysRS binds to capsid^{Gag} for LysRS packaging, but Vpr inhibits the enzymatic activity of LysRS	159, 367–369, 372, 373
Gag-AIP1-Nef	Budding	Gag and Nef recruit AIP1 to promote viral budding	375–378
NC^{Gag}-Tsg101/AIP1-p6^{Gag}	Budding	NC^{Gag} and p6^{Gag} recruit Tsg101 and AIP1 to promote viral budding	378, 385–390
Vif-A3G-integrase	Budding	A3G binds to integrase for prohibiting proviral DNA formation, but Vif induces A3G degradation	33–43, 395
Vif-MAPK/HCK-Nef	Budding	Nef activates the HCK pathway to downregulate cell surface receptors, but Vif counteracts HCK-mediated inhibition of viral release; MAPK phosphorylates Vif, but Nef inhibits the kinase activity of MAPK	407, 408, 415–421
Vpu-CD4-GP120^{Env}	Budding	CD4 prevents GP120 transport for viral budding, but HIV-1 Vpu induces CD4 degradation	14, 15, 427–432, 435, 436, 450
Vpu-tetherin/CD4-Nef	Budding	HIV-1 Vpu and Nef antagonize tetherin and CD4 to promote viral budding	456–463, 465–468, 475
Vpu-CK2-Rev	Budding	Rev promotes CK2 activity, which phosphorylates HIV-1 Vpu for CD4 degradation	453, 482–484
Vpu-UBP-matrix^{Gag}	Budding	UBP mediates a functional association between HIV-1 Vpu and matrix^{Gag}	486–490

[a] Protein association indicates that two HIV proteins either independently or dependently interact with a third molecule (e.g., CD4 or dsDNA). Note that two HIV proteins in a protein association are not necessary to construct a structural complex or to undertake biological activities at the same time during the viral life cycle. A3G, APOBEC3G.

tion assays (110–113). The binding of HIV-1 nucleocapsid to RT contributes to the increased production of long proviral DNA transcripts (114, 115). At the early stage of reverse transcription, HIV-1 nucleocapsid interacts with RT to facilitate the annealing of primer tRNA^{Lys3} onto viral genomic RNA (110, 116). At the final stage of reverse transcription, a 99-nucleotide DNA flap is established in the center of the proviral DNA genome to mediate the nuclear import of the HIV-1 genome (117, 118). The construction of this central flap requires nucleocapsid chaperone activity, RT-mediated DNA synthesis, and the critical interaction between nucleocapsid and RT (117). Multiple activities of nucleocapsid take place during reverse transcription. (i) The nucleic acid-binding and chaperoning properties of nucleocapsid stabilize the RT-DNA complex to promote reverse transcription (119–121). The chaperoning activity of nucleocapsid also protects HIV-1 RNA from degradation induced by the RNase H domain of RT (122). Moreover, nucleocapsid improves the stability of RT-substrate complexes by reducing dissociation rate constants (120). (ii) Nucleocapsid not only promotes the RT strand transfer reaction (112, 122–124) but also increases RT processivity and primer extension at specific DNA template sites (125). The binding of nucleocapsid to RT counteracts the decreased strand transfer efficiency of RT mutants (113). (iii) At the early stage of HIV-1 reverse transcription, nucleocapsid can destabilize the stem-loop structure of the primer-binding site that governs the initiation-to-elongation transition and causes the major pauses during primer extension (126). Moreover, nucleocapsid inhibits primer extension prior to the formation of the RT-primer/template-deoxynucleoside triphosphate (dNTP) structural complex (125). In line with this evidence, mutations at the zinc finger domains of nucleocapsid cause premature reverse transcription (127). (iv) The excision repair activity of RT, a mechanism by which RT corrects mismatches at the cDNA polymerization site, is stimulated by nucleocapsid (119). The nucleocapsid-mediated annealing of the primer template promotes RT activity by reducing the rate of incorrect nucleotide incorporation (128).

Regarding the interaction domains, two zinc finger domains in nucleocapsid may interact with RT (110), and they are crucial for the efficient unfolding of highly structured RNA and DNA intermediates during the RT strand transfer reactions (124). HIV-1 nucleocapsid improves the RNase activity of the RNase H domain in HIV-1 RT (129), while RT regulates the nucleocapsid architecture to coordinate HIV-1 preintegration processes (130). The exact interaction domains in RT are yet to be discovered by future studies.

RT-Vif Interaction

As a component of the RTC (94), Vif has been detected to interact with RT by using GST pulldown assays (131). During the early stage of reverse transcription, the RT-Vif interaction stimulates primer annealing and increases the polymerization rate (132, 133). Multiple activities of Vif take place during reverse transcription. (i) Vif can modulate nucleic acid components in the viral genomic RNA and $tRNA^{Lys3}$ to promote efficient reverse transcription (134), although this process happens mainly as an early event after viral entry (135). (ii) Vif not only stimulates the formation of loose HIV-1 genomic RNA dimers but also collaborates with nucleocapsid to enhance single-stranded DNA (ssDNA) synthesis (133). At an early stage of reverse transcription, Vif inhibits the hybridization of $tRNA^{Lys3}$ and prevents the nucleotide-mediated formation of RNA dimers (133). (iii) The stable accumulation of HIV-1 reverse transcripts is mediated by Vif (136). Vif-defective mutants cause impaired DNA synthesis as well as reduced RT activity in nonpermissive cells (137, 138). However, Vif neither exerts an impact on genomic RNA dimerization nor affects the stability of the RNA dimer linkage (135).

Regarding the interaction domains, the C-terminal domain of Vif (positions 161 to 164) physically interacts with RT to stimulate reverse transcription (131). To our knowledge, RT functional domains that interact with Vif remain unclear. Additional studies are also required to verify the reproducibility of the RT-Vif interaction.

RT-Tat Interaction

The direct interaction between RT and Tat has been detected by GST pulldown assays, coimmunoprecipitation assays, and mammalian two-hybrid assays (139). An HIV-1 Tat mutant called nullbasic, whose entire arginine-rich domain is replaced by either glycine or alanine, has also been proven to interact with RT by using coimmunoprecipitation assays, pulldown assays, and biolayer interferometry assays (140). As an antiviral protein, nullbasic reduces viral core stability to prevent HIV-1 reverse transcription (140). Although Tat is dispensable for reverse transcription, Tat in complex with RT stimulates viral DNA synthesis (139). In comparison with its activity in gene expression, Tat uses distinct mechanisms to regulate HIV-1 reverse transcription (141). First, the nucleic acid chaperone activity of Tat not only promotes the placement of $tRNA^{Lys3}$ onto viral RNA but also suppresses nonspecific DNA polymerization (142). Second, HIV-1 Tat prevents the synthesis of deleterious DNA products and interrupts DNA polymerization during the late stages of reverse transcription (143). Third, Tat acts cooperatively with nucleocapsid to promote nucleic acid annealing for the RT strand transfer reaction (144). Overall, Tat contributes to efficient reverse transcription, as HIV-1 strains lacking Tat are defective in endogenous assays of reverse transcription (145).

The RT-Tat interaction domains in the RT partner are mapped to the p51 subunit by GST pulldown and immunoprecipitation assays and to the p51 and p66 subunits by mammalian two-hybrid assays (139). The basic region of Tat (positions 44 to 61) may promote the RNA-annealing reaction by HIV-1 RT (144, 146). Two cysteine-rich domains of Tat (positions 21 to 39 and 40 to 47) suppress DNA elongation during reverse transcription (142). By altering the positive-charge distribution, the acetylation of Tat residues K28, K50, and K51 can regulate the activity of Tat in reverse transcription and transcriptional activity (146, 147). Overall, the basic domain of Tat plays a role in efficient reverse transcription, but the exact RT domains that interact with Tat remain unclear. Additional studies are still required to verify the reproducibility of the RT-Tat interaction.

RT-Nef Interaction

HIV-1 Nef can physically interact with RT according to GST pulldown assays, coimmunoprecipitation assays, and *in vitro* binding assays (148, 149). In fact, Nef can stimulates proviral DNA synthesis during reverse transcription (150). Being independent of its binding to viral RNA, Nef increases the binding affinity of HIV-1 RT for viral RNA (148). In the absence of Nef, RT generates 5- to 10-fold-fewer DNA products (151). Regarding protein interaction domains, data from mutagenesis analyses suggest that the p51 unit of RT may interact with the disorder loop in the C-terminal domain of Nef (positions 154 to 172) (148). Although HIV-1 Nef may play a role during reverse transcription, future studies are still needed to verify the reproducibility of the RT-Nef interaction.

Protease-Tat Interaction

Data from cell-free and cell culture assays suggest that HIV-1 protease cleaves Tat (152). This protease-mediated cleavage requires the basic domain of Tat (positions 49 to 57) (152). The Tat motif $R^{49}KKR^{52}$ plays a critical role in modulating HIV-1 reverse transcription (152). Moreover, a Tat mutant harboring a single mutation, Y47N, near the protease cleavage site can downregulate Tat-stimulated reverse transcription, suggesting that the protease-mediated cleavage of Tat influences Tat-enhanced reverse transcription (152). Future studies are still required to verify protease-mediated cleavage on HIV-1 Tat, as it has been reported in only a single study.

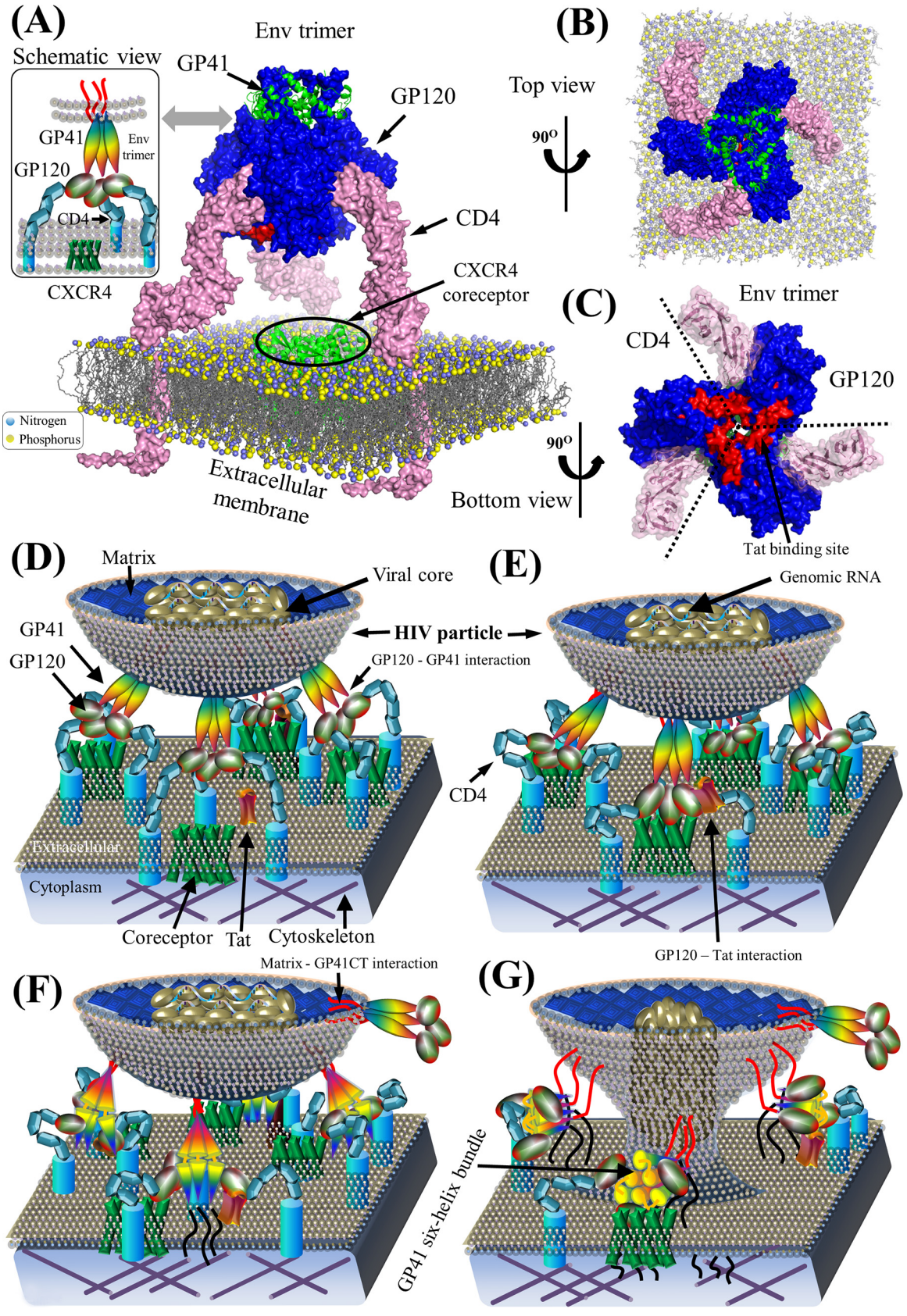
RT-$tRNA^{Lys3}$-Vpr Association

Although both RT and Vpr are colocalized in the RTC, the PIC, and the viral core (153, 154), a direct interaction between RT and Vpr has not been reported to our knowledge. Based on HIV-host protein interactions, cellular primer $tRNA^{Lys3}$ physically interacts with RT (155–158) and Vpr (159). During reverse transcription, RT initiates minus-strand DNA synthesis from the 3′ end of primer $tRNA^{Lys3}$ (160). To influence the initiation of reverse transcription, Vpr interacts with $tRNA^{Lys3}$ and prohibits the LysRS-mediated aminoacylation of $tRNA^{Lys3}$ (159). Of interest, $tRNA^{Lys3}$ is packaged into HIV-1 virions with ~20 molecules per virion (161).

For efficient DNA synthesis, the thumb subdomain in the p66 unit of RT may interact with the anticodon loop in $tRNA^{Lys3}$ (155). The RT connection domain may take part in $tRNA^{Lys3}$ annealing but not in $tRNA^{Lys3}$ packaging (156). The $V^{241}QPI^{244}$ peptide in the cross-link between the thumb and the palm subdomains of RT (Fig. 6A) may interact with primer $tRNA^{Lys3}$ (158). Despite the fact that interaction domains in Vpr are yet to be resolved, peptides derived from two Vpr regions (positions 57 to 71 and 61 to 75) can interact with RT to inhibit HIV-1 reverse transcription (162).

VIRAL INTEGRATION

After HIV reverse transcription, the RTC reorganizes into the PIC in the cytoplasm (Fig. 7). Although the exact composition of the PIC remains debated (163), the PIC is likely comprised of cellular



cofactors, dsDNA, integrase, RT, matrix, nucleocapsid, Vpr, and a small amount of capsid (96, 164–166). During viral integration, a series of actions take place (24–26, 167, 168). The first action is 3′-end processing. In the cytoplasm, the viral integrase tetramer removes 2 nucleotides at each 3′ end of dsDNA to generate a reactive intermediate that contains a 3′-hydroxyl group (168). The second action is nuclear import. The PIC-containing dsDNA is imported from the cytoplasm to the nucleus through nucleus pore complexes (168). The third step is nuclear localization. The PIC is localized to host chromosomal domains with high transcriptional activity (24). This process is assisted by cellular cofactors such as lens epithelium-derived growth factor (LEDGF)/p75, a cellular transcriptional coactivator serving as a tethering protein between the PIC and host chromosomes (167). The fourth action is the strand transfer reaction. Viral dsDNA is inserted into host chromosomes through the integrase strand transfer reaction (168). The final action is gap repair. Unpaired regions between HIV and host dsDNA are repaired under the assistance of cellular cofactors (24).

The mechanisms of PIC nuclear import, preintegration transcription, and integration-associated host proteins (e.g., LEDGF/p75) have been reviewed elsewhere (24–26, 167–170). Here, we focus on physical interactions and functional associations between these HIV proteins that take place during viral integration.

Integrase-RT Interaction

HIV integrase has been determined to physically interact with RT by using GST pulldown assays, coimmunoprecipitation assays, dot blot assays, NMR spectroscopy, and surface plasmon resonance analyses (101–107). Two functions of the integrase-RT interaction have been proposed. First, RT in the PIC inhibits both the 3′-end endonuclease and the strand transfer activity of integrase (101, 103). Second, RT can inhibit the DNA disintegration activity of integrase before viral integration, although HIV-1 integrase may promote RT activity during reverse transcription (171). Note that DNA disintegration is a reverse reaction of viral integration that releases viral dsDNA and repairs the continuity of host chromosomes (172). Overall, RT can efficiently regulate the activity of integrase through the integrase-RT interaction.

Regarding the interaction domains, the CTD of integrase is necessary and sufficient for the interaction with RT (102, 106). For instance, amino acid substitutions (W243E, V250E, and K258A) at the integrase CTD severely impair the integrase-RT interaction (102). As for the interaction domains in HIV-1 RT, the finger-palm domain and the C-terminal half of the connection domain of the RT heterodimer may interact with the integrase CTD (108). In terms of different interaction domains reported during viral integration and reverse transcription, additional analyses are still needed to verify whether these differences are detected in different cell lines, HIV-1 strains, or conformation rearrangements adapted for different activities of the RTC and the PIC.

Integrase-Rev Interaction

HIV-1 Rev has been found to physically interact with the integrase dimer or tetramer by using GST pulldown assays and coimmunoprecipitation assays (173–175). It is known that integrase interacts with the cellular LEDGF/p75 protein (168, 176, 177). Experimental evidence suggests that Rev may disrupt the interaction between integrase and LEDGF/p75, a mechanism that inhibits premature viral integration before the nuclear localization of viral dsDNA (173, 178). At the postintegration stage, Rev expressed at the pretranscription processing step can prevent the nuclear import of integrase through the Rev-integrase interaction, thereby limiting the massive number of copies of viral DNA integrated into host chromosomes (175, 179). Since increased integration has been postulated to cause excessive cell death, Rev thus protects HIV-1-infected cells from premature cell death (175).

Regarding the interaction domains, two Rev domains (positions 13 to 23 and 53 to 67) may interact with the central regions of integrase (positions 118 to 128 and 66 to 80) (173). Interestingly, Rev-derived peptides (positions 13 to 23 and 53 to 67) inhibit the activity of integrase, whereas integrase-derived peptides (positions 66 to 80 and 118 to 128) rescue the Rev-mediated inhibitory effect (180).

Integrase-Matrix Interaction

HIV-1 integrase has been identified to interact with matrix by using coimmunoprecipitation assays (181). The integrase-matrix interaction promotes the nuclear import of the PIC in nondividing cells such as macrophages (181). Although viral integrase and matrix are components of the PIC, the entire matrix is dispensable for viral nuclear import (182). Regarding the interaction domains, the catalytic core domain of integrase (positions 50 to 212) may bind to matrix, while C-terminal tyrosine phosphorylation of matrix is crucial for the integrase-matrix interaction (181). Replacing tyrosine with phenylalanine at matrix position 132 can block PIC nuclear import (181). Independent analyses are still required to verify the reproducibility of the integrase-matrix interaction.

Matrix-Vpr Interaction

HIV-1 matrix has been found to interact with Vpr by using yeast two-hybrid assays and coimmunoprecipitation assays (183). As

FIG 5 Env structure complex and schematic model of HIV-1 pairwise protein interactions during viral entry. (A) Structural model of a prefusion HIV-1 Env spike associated with CD4 on the extracellular membrane. Surface representations of GP120, GP41, and CD4 proteins are shown in blue, green, and pink, respectively. Lipid bilayers of the extracellular membrane (606) are shown at the bottom, where nitrogen and phosphorus are indicated by blue and yellow spheres, respectively. The crystallized structure of the CXCR4 coreceptor in green is placed in the center across the extracellular membrane. Red areas on the GP120 surface illustrate the Tat-binding site (86, 89). Table S1 in the supplemental material provides a list of PDB accession numbers used for our structural visualization. PyMOL V1.7 visualization software was used (see http://www.pymol.org/). (B) Top view of a prefusion HIV-1 Env spike in complex with CD4 on the extracellular membrane. (C) Bottom view of the prefusion HIV-1 Env spike in complex with CD4. GP120 subunits within the trimeric Env spike bind to CD4. Red areas indicate Tat-binding sites. (E) Schematic view of the binding of Env to CD4 and coreceptors for viral attachment to the host membrane. GP120 on the mature virion surface interacts with CD4 to induce the aggregation of CD4 and chemokine coreceptors (e.g., CCR5 and CXCR4) (607, 608). Thereafter, GP120 binds to chemokine coreceptors on the host membrane. (F) Construction of GP41 six-helix bundles. Interactions between GP120 and chemokine coreceptors induce conformation rearrangements in Env spikes, which expose GP41 to construct the six-helix bundles (20). (G) Viral entry. GP41 six-helix bundles pull the extracellular membrane to create a fusion pore, which might consist of 1 to 7 Env spikes depending on divergent HIV strains (61, 62). The viral core in the HIV particle is then injected into the host cytoplasm by entering the newly created fusion pore. Note that protein shapes do not represent the exact protein structures, nor are the protein sizes to scale.

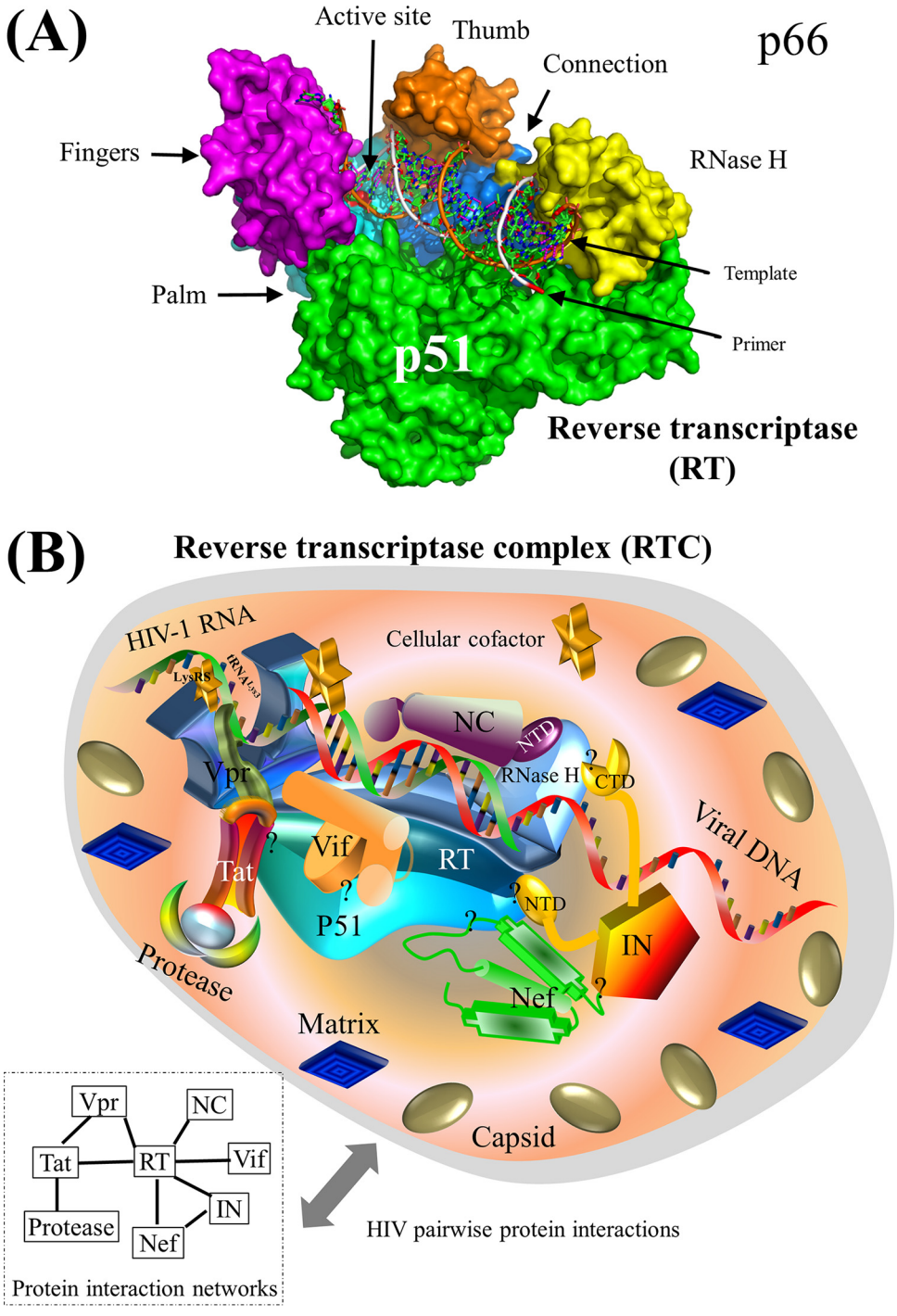


FIG 6 Surface representation of HIV-1 reverse transcriptase and schematic model of the HIV-1 RTC. (A) Surface representation of HIV-1 RT (PDB accession number 3KLG). Two major subunits, p51 and p66, are annotated. The p66 subunit consists of fingers, thumb, palm, connection, and RNase H domains (609). HIV-1 RNA/DNA is shown in the middle, and the active site of RT is mapped to the 3′ end of the DNA located between the fingers and thumb domains of HIV-1 RT. See Movie S1 in the supplemental material for a structural movie of HIV-1 RT. (B) Schematic model of HIV-1 pairwise protein interactions in the RTC. The HIV-1 RTC consists of RT, protease, integrase, matrix, capsid, nucleocapsid, Vif, Tat, Nef, Vpr, and many cellular proteins, although the exact composition of the RTC remains debated (93–95). HIV-1 capsid and a subset of phosphorylated matrix are weakly associated with the RTC (95, 96). Seven HIV-1 pairwise protein associations, including the RT-integrase, RT-nucleocapsid, RT-Vif, RT-Tat, RT-Nef, and protease-Tat interactions as well as the RT-$tRNA^{Lys3}$-Vpr association, are mapped (Tables 1 and 3). Cellular cofactors (e.g., eEF1A), marked by yellow stars, may interact with the RTC to facilitate HIV-1 reverse transcription (610). Localization of the RTC in the cytoplasm is mediated by the interaction between HIV-1 matrix in the RTC and the actin cytoskeleton (611), although only a small subset of matrix is present in the RTC (95). A protein-protein interaction network is shown at the bottom left to demonstrate the physical protein interactions. Question marks indicate unclear interaction domains. Note that protein shapes do not represent the exact protein structures, nor are the protein sizes to scale.

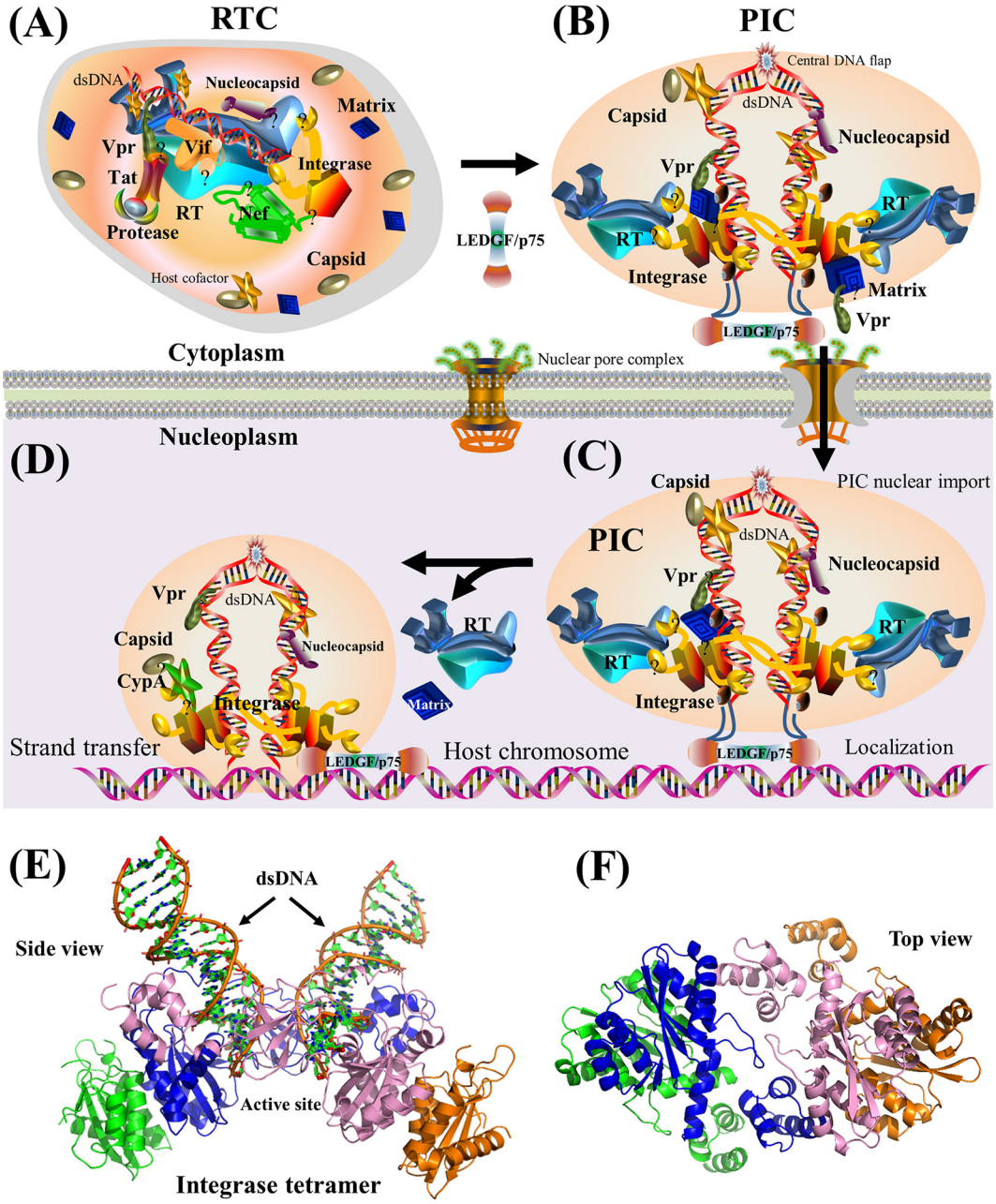


FIG 7 Schematic model of viral integration and cartoon representation of viral integrase. The HIV-1 PIC is comprised of cellular cofactors, viral dsDNA, integrase, RT, matrix, nucleocapsid, Vpr, and a small amount of capsid (96, 164–166), although the exact composition of the PIC remains debated (163). (A) Viral uncoating. The RTC turns into the PIC with the recruitment of host factors (e.g., LEDGF/p75). During this process, Nef, Tat, and most capsid proteins are dissociated from the PIC (169). (B) Nuclear import. The PIC is imported from the cytoplasm to the nucleoplasm. Vpr physically interacts with the nuclear pore complex for PIC nuclear import (612). The formation of the central DNA flap promotes viral uncoating at the nuclear pore (613). Although it remains debated, a small amount of capsid is associated with the PIC to enhance its nuclear import (96). At the late stages of viral integration, Rev may interact with integrase to prevent the nuclear import of the overexpressed PIC (179). (C) Chromosomal localization. Assisted by cellular proteins such as LEDGF/p75, HIV-1 dsDNA in the PIC is tethered to the host chromosome (170). (D) Integration. HIV-1 dsDNA is integrated into host chromosomes by viral integrase (168). (E) Cartoon representation of a prototype foamy virus integrase in complex with dsDNA (PDB accession number 3OY9). The active site of integrase is shown in the middle. See Movie S2 in the supplemental material for a structural movie of viral integrase. (F) Cartoon representation of the HIV-1 integrase tetramer in the absence of dsDNA (PDB accession number 1K6Y). Four subunits of the integrase tetramer are shown in green, blue, pink, and orange, respectively. For the schematic models in panels A to D, the protein shapes do not represent the exact protein structures, nor are the protein sizes to scale. Question marks indicate that interaction domains remain unclear.

nucleophilic proteins, HIV-1 matrix and Vpr collaboratively improve the stoichiometry of nucleophilic components in the PIC and promote PIC nuclear import in nondividing cells (184). Although Vpr-mediated nuclear export is dispensable for viral packaging (185), HIV-1 Vpr promotes the nuclear import of the PIC in macrophages (186). In fact, HIV-1 Vpr in the cytoplasm is transported into the nucleus by hijacking cellular proteins such as importin alpha (186). As described previously, matrix also takes part

in nuclear import due to its interaction with HIV-1 integrase (181). Regarding the interaction domains, the C-terminal domain of matrix (positions 88 to 132) may interact with Vpr (183), but the interaction domains in Vpr remain unclear. Future studies are still required to verify the reproducibility of the matrix-Vpr interaction.

Integrase-Nef Interaction

The physical interaction between integrase and Nef has consistently been detected by using yeast two-hybrid assays, coimmunoprecipitation assays, and GST pulldown assays (149). Although biological functions of this interaction remain unclear, it is speculated that Nef may take part in HIV-1 reverse transcription and integration (149). More studies are required to investigate the activities of this interaction as well as the binding domains. Additional analyses are still required to verify the reproducibility of the integrase-Nef interaction, as it has been reported in only a single study.

Integrase-dsDNA-Vpr Association

HIV-1 integrase, Vpr, and dsDNA are key components of the HIV PIC (Fig. 7). During viral integration, it is known that integrase inserts viral dsDNA into host chromosomes (24). As the most abundant viral protein in the PIC (187), HIV-1 Vpr promotes the nuclear localization of viral dsDNA during the nuclear import of the HIV-1 PIC (184, 188). To enhance PIC nuclear import, HIV-1 Vpr acts as a DNA architectural protein to bridge two or more DNA helices into synaptic and stretched nucleofilaments (187). The binding of HIV-1 Vpr to DNA also induces double-strand breaks in chromosomal DNA, which might influence viral integration (189). Moreover, full-length Vpr and its C terminus (positions 52 to 96) not only stimulate the strand transfer reaction but also enhance the binding of integrase to viral dsDNA (190). Interestingly, Vpr-derived peptides (positions 57 to 71 and 61 to 75) can inhibit the activity of HIV-1 integrase (162). Overall, Vpr may promote integrase activity via its interaction with dsDNA, although a direct interaction between integrase and Vpr remains unclear.

Integrase-dsDNA-Nucleocapsid Association

Although a direct interaction between integrase and nucleocapsid has not been reported, the integrase-dsDNA-nucleocapsid structural complex plays multiple roles during viral integration. (i) The chaperone activity of nucleocapsid on viral DNA enhances HIV-1 integration (191). Specifically, the zinc finger domains of nucleocapsid not only stabilize the binding of integrase to viral dsDNA but also complement DNA binding to promote the integrase strand transfer reaction (192). (ii) HIV-1 nucleocapsid can promote coupled integration by >1,000-fold under *in vitro* conditions (193). During viral DNA integration, viral integrase takes part in the coupled joining that merges two ends of the viral genome into the host genome (193). (iii) In the presence of Mg^{2+}, a high concentration of viral integrase is required for HIV-1 integration (194). For an efficient DNA strand transfer reaction, nucleocapsid counteracts this defect by keeping a low concentration of integrase in the presence of Mg^{2+} (194). Overall, nucleocapsid interacts with viral dsDNA to promote integrase activities during viral integration.

Integrase-TNPO3/CypA-Capsid Association

Although a direct interaction between capsid and integrase has not been reported, transportin 3 (TNPO3, transportin-SR2, or TRN-SR2) interacts with viral integrase and capsid to facilitate the nuclear transport of the viral PIC (195–198). As a member of the importin-β family, TNPO3 recognizes serine/arginine-rich repeats within precursor mRNA splicing factors and transports these factors from the cytoplasm to the nucleus (199). Regarding the interaction domains, it has been reported that TNPO3 interacts with amino acid positions in the integrase CTD (e.g., R262 to K264, K266, and R269) (198). HIV-1 integrase mutants with R262A and K264A mutations display a weak interaction with TNPO3, showing a 2.3-fold-lower affinity than that of the wild-type integrase (200). Although it is not a major determinant of HIV-1 nuclear import, the integrase-TNPO3 interaction may take place when the PIC enters the nucleus but before viral integration (201, 202). Other studies also suggest that viral capsid, not integrase, dictates the TNPO dependency of PIC nuclear import (195, 201).

Cyclophilin A (CypA) is an important cellular peptidyl-prolyl isomerase that participates in the uncoating of viral core (203, 204). CypA physically interacts with viral capsid (204–206), while viral integrase is required to maintain the physical interaction between capsid and CypA (203). Owing to the capsid-CypA interaction, HIV-1 capsid can be either stabilized or destabilized by CypA (207, 208). On the one hand, viral capsid is stabilized by CypA when it travels to the nuclear pore in the cytoplasm (205). On the other hand, viral capsid escapes from CypA dependence through conformational dynamics (206). Accumulated evidence also implies a direct association between HIV-1 capsid and integrase, because capsid mutants (Q63A and Q67A) exert a deleterious effect on viral integration (164). Moreover, the presence of integrase mutants (e.g., C130S) induces the degradation of capsid in the cytoplasm, thus decreasing viral core stability (203).

Overall, viral capsid and integrase are associated with cellular proteins (e.g., TNPO3 and CypA) in order to facilitate PIC nuclear import and viral integration.

VIRAL TRANSCRIPTION AND TRANSLATION

Two different HIV transcription pathways have been observed before and after viral dsDNA integration. (i) A small amount of regulatory proteins (Rev, Tat, and Nef) can be synthesized from unintegrated viral DNA, a process called preintegration transcription (25, 209) (Fig. 3). These synthesized viral proteins interact with cellular machineries to regulate viral production at subsequent stages of the HIV life cycle (Fig. 8). For instance, Rev transports viral RNAs from the nucleus to the cytoplasm (210). (ii) A large number of viral mRNAs are produced by cellular microRNA (miRNA) machineries, which synthesize mRNA from viral dsDNA integrated into host chromosomes (27). Viral mRNAs are then processed (polyadenylation, methylation, capping, and splicing) for protein maturation (211). Posttranslational modifications of viral proteins (e.g., phosphorylation, methylation, and acetylation) are also essential during this process (147, 212).

Previous studies have reviewed mechanisms of HIV transcription and translation (27–29), Rev-mediated nuclear export (213), Gag-mediated nuclear localization (214), and interactions between HIV proteins and cellular transcription factors (28, 29, 215). Here, we focus on physical interactions and functional associations between HIV-1 proteins, which play important roles in viral transcription and translation.

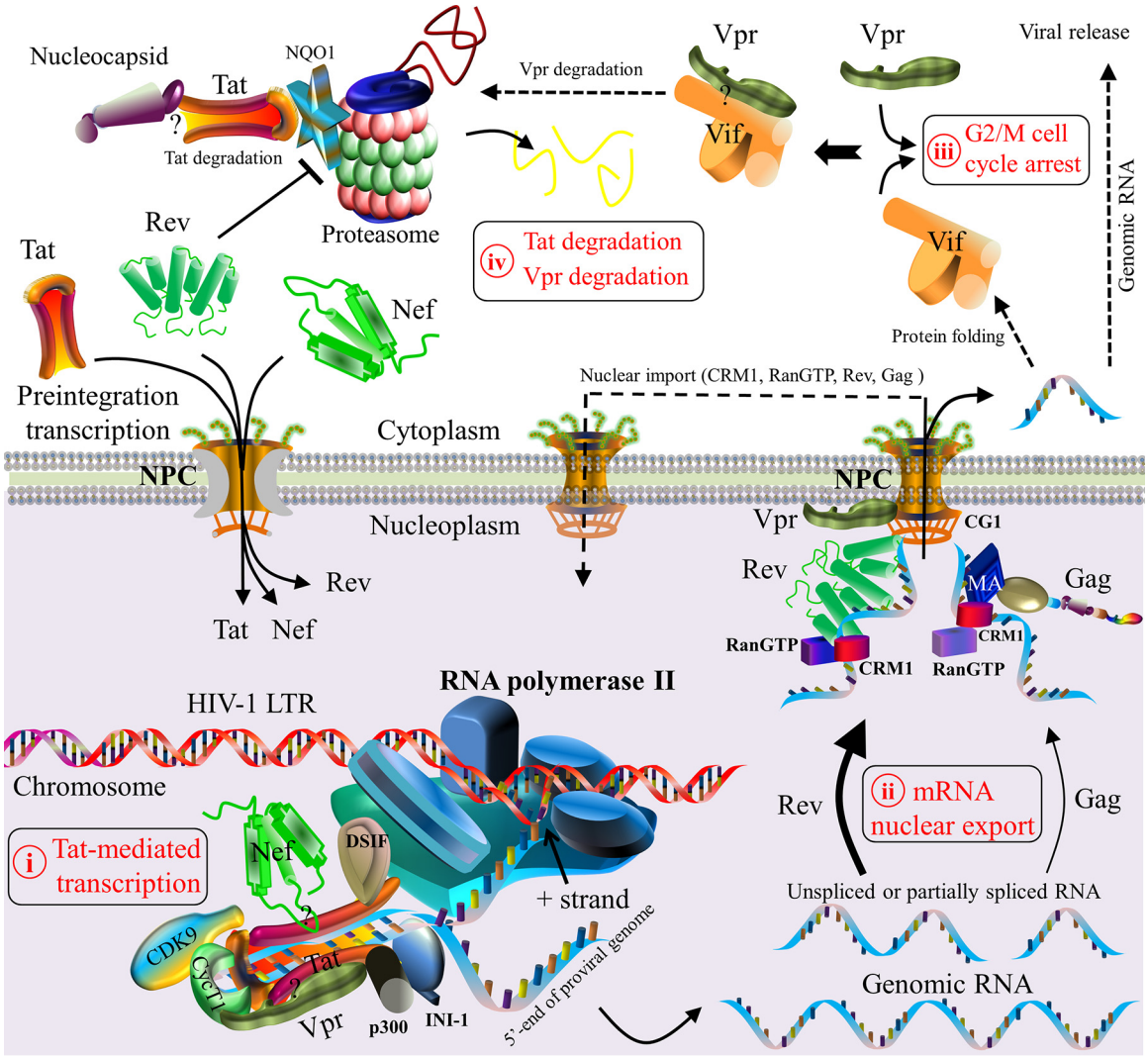


FIG 8 Schematic model of HIV-1 protein interactions during viral transcription and translation. Five steps are described. (i) HIV-1 Tat initiates viral transcription by its interaction with the TAR of viral RNA, which is a regulatory element located downstream of the HIV-1 LTR (212). Tat subsequently recruits the subunits of the positive transcription elongation factor (e.g., cyclin T1 [CycT1] and cyclin-dependent kinase 9 [CDK9]) to construct a transcription complex (212). This complex activates the kinase CDK9 for the hyperphosphorylation of RNA polymerase II, which interacts with Tat and other host factors (e.g., DSIF [5,6-dichloro-1-β-D-ribofuranosylbenzimidazole sensitivity-inducing factor] [614]) to produce viral genomic mRNAs (615, 616). Genomic mRNAs are spliced thereafter. Although many host proteins take part in viral transcription (23), only CDK9, cyclin T1, p300, DSIF, INI1, and RNA polymerase II are shown. (ii) Nuclear export of newly synthesized viral RNAs is accomplished by either Rev- or Gag-mediated pathways (239–242). In the former case, Rev recruits cellular factors (e.g., CRM1 and RanGTP) to export viral genomic RNAs as well as incompletely spliced and unspliced mRNAs from the nucleoplasm to the cytoplasm (30, 213). The binding of Vpr to the nuclear pore complex (NPC) is predominantly localized in the nuclear envelope of the nucleus (247, 248). In the latter case, RNA nuclear export is activated by the nuclear export signal (NES) of matrix^{Gag}, which interacts with CRM1 (214, 242). The nuclear export of viral RNA allows viral protein maturation in the cytoplasm and cellular compartments. CRM1, RanGTP, Rev, and Gag are then imported back to the nucleus (213, 214, 242). (iii) HIV-1 Vif and Vpr, both of which are expressed in the cytoplasm, can independently trigger G_2/M cell cycle arrest (233). Vif may also interact with Vpr to mediate the degradation of Vpr and to reduce Vpr-induced cell cycle arrest (232). (iv) At the late stage of viral transcription, the proteasomal degradation of Tat is induced by viral nucleocapsid (228). Moreover, Rev induces Tat degradation by downregulating the expression level of the cellular protein NQO1 (216). Question marks indicate unclear interaction domains. Note that protein shapes do not represent the exact protein structures, nor are the protein sizes to scale.

Tat-Rev Interaction

The direct interaction between HIV-1 Rev and Tat has been detected by two-hybrid assays, pulldown assays, and coimmunoprecipitation assays (216). The nuclear export signal region of HIV-1 Rev (Fig. 2) takes part in the proteasomal degradation of cytoplasmic Tat at the posttranslational level, leading to a significant decrease of HIV-1 gene expression (216). The Rev-mediated downregulation of Tat might be associated with HIV-1 latency, because the decrease of the intracellular level of Tat below a critical threshold potentially marks the rise of HIV-1 latency (216). Moreover, the regulatory proteins Rev and Tat shuttle between the nucleus and the cytoplasm to interact with various cellular factors (4, 216). HIV-1 Rev and Tat expressed via viral preintegration transcription (Fig. 3) are frequently targeted by human cytotoxic T lymphocytes for the immune control of viral infections (217).

Although the Tat-Rev interaction does not induce Rev-mediated Tat degradation, Rev causes Tat degradation in the cytoplasm by downregulating the level of a host protein called NAD(p)H:quinine oxidoreductase 1 (NQO1) (216). A host protein called DEAD box RNA helicase (DDX1) has also been reported to interact with both HIV-1 Rev and Tat (218). During nuclear export, Rev interacts with DDX1 to promote Rev multimerization on the Rev response element (RRE) of viral mRNA (219). Moreover, an HIV-1 Tat mutant, called nullbasic, interacts with DDX1 to disrupt the subcellular localization of Rev, thereby decreasing the expression of Rev-dependent viral mRNA (218). To counteract this defect, wild-type Tat interacts with DDX1 to restore the Rev-mediated export of viral mRNA (218). Regarding the interaction domains, the nuclear localization signal (positions 35 to 50) of Rev is vital for the Tat-Rev interaction (216), whereas interaction domains in Tat remain unclear. Additional studies are still required to verify the reproducibility of the Tat-Rev interaction.

Tat-Vpr Interaction

Data from GST pulldown and coimmunoprecipitation assays suggest that HIV-1 Vpr physically interacts with Tat and cyclin T1 in the nucleus (220). Tat is a regulatory protein known for its interaction with positive transcription elongation factor b (pTEFb), a structural complex consisting of cyclin-dependent kinase 9 (CDK9) and cyclin T1 (221, 222). During viral transcription, a tertiary complex, Tat-Vpr-pTEFb, is constructed to promote the superactivation of the HIV-1 long terminal region (LTR), leading to increased transcriptional activity (220).

Regarding the interaction domains, Vpr may interact with the Tat domain within amino acid positions 50 to 67 (220). Moreover, a single substitution, R73S, in Vpr severely reduces the Tat-induced transcription of the HIV-1 LTR, suggesting a key role of Vpr R73 in modulating Tat activity (220). The exact interaction domains in Vpr are yet to be clarified. More studies are still needed to verify the Tat-Vpr interaction, because this interaction has been reported in only a single study.

Tat-Nef Interaction

The direct interaction between HIV-1 Tat and Nef has been identified by using coimmunoprecipitation assays, GST pulldown assays, and transient-transfection assays (223). Colocalized in the nucleus, both Tat and Nef can be expressed during preintegration transcription (Fig. 3). First, Nef induces many host factors (e.g., CDK9, Tat-SF1, and IRF2) to promote Tat-mediated transcriptional activity (224). Second, Nef-mediated signaling can enhance Tat-mediated transcriptional activity via an extracellular signal-regulated kinase (ERK) mitogen-activated protein kinase (MAPK)-dependent pathway (225). Third, Nef promotes Tat-mediated transcription via the heterogeneous nuclear ribonucleoprotein K (hnRNP-K)-nucleated signaling complex (226). Note that hnRNP-K plays essential roles in transcriptional processes and molecular interactions (227). Overall, Nef exerts an impact on Tat-mediated transcription either by direct interaction or by signaling pathways mediated via cellular cofactors (223–226). The exact protein domains that mediate the Tat-Nef interaction remain unclear. Future studies are still required to verify the Tat-Nef interaction and its functions.

Tat-Nucleocapsid Interaction

The direct interaction between Tat and nucleocapsid has been detected by using yeast two-hybrid assays, GST pulldown assays, coimmunoprecipitation assays, and subcellular colocalization assays (228). For HIV-1 and HIV-2, both Tat and nucleocapsid are chaperone proteins that mediate the proper folding of viral RNA (229, 230). Interestingly, the proteasomal degradation of Tat is induced by viral nucleocapsid in a ubiquitin-independent manner, subsequently reducing Tat-mediated transcription at the late stage of viral transcription (228). In the absence of Tat, nucleocapsid is localized predominantly in the cytoplasm (228), even though nucleocapsid can shuttle from the cytoplasm to the nucleus (231). The exact interaction domains in Tat and nucleocapsid remain unclear. Additional studies are still required to verify the Tat-nucleocapsid interaction, because it has been reported in only a single study.

Vif-Vpr Interaction

HIV-1 Vif may interact with Vpr according to coimmunoprecipitation assays (232). HIV-1 Vpr and Vif share common activities during the viral life cycle: (i) both HIV-1 proteins independently cause T-cell cytopathicity (233), (ii) they can promote viral infections by the induction of cell cycle arrest at the G_2/M phase in dividing cells (234–237), and (iii) HIV-1 Vpr and Vif downregulate the antiviral cellular factor APOBEC3G through the proteasomal degradation pathway (238). On the other hand, Vif may interact with Vpr to mediate the degradation of Vpr via the ubiquitin and proteasome pathways (232). It has been speculated that this interaction may modulate Vpr activity in order to decrease the accumulation of HIV-infected cells at the stage of G_2/M cell cycle arrest (232). Experimental evidence also suggests that the elimination of both the *vif* and *vpr* genes from the HIV-1 genome, but not each gene individually, prevents cell death and G_2/M cell cycle arrest of HIV-infected cells (233). To our knowledge, the exact protein domains that mediate the Vpr-Vif interaction remain unclear. More studies are still required to verify the Vif-Vpr interaction, as it has been reported in only a single study.

Rev-CRM1-Matrix^{Gag} Association

HIV-1 Rev and matrix^{Gag} physically interact with a cellular protein called chromosome region maintenance 1 (CRM1) to export viral RNA via the Rev-mediated or the Gag-mediated export pathways (239–242). As a major pathway, Rev recruits CRM1, RanGTP, and other host proteins (e.g., DDX3) to export viral mRNA from the nucleus to the cytoplasm (213, 243). The CRM1-Rev interaction has been demonstrated by pulldown assays, mammalian two-hybrid assays, gel mobility shift assays, and protein footprinting assays (239, 240). During the early stage of HIV-1 infection, the nuclear export signal of Rev binds to CRM1 and other cellular factors, leading to the dynamic trafficking of Rev between the nucleus and the cytoplasm (239, 240).

The CRM1-matrix^{Gag} interaction, identified by two-hybrid assays, paves the way for the Gag-mediated nuclear export pathway (242). Matrix^{Gag} harbors one nuclear export signal (NES) (positions 18 and 22) (242) and two nuclear localization signals (NLSs) (positions 24 to 31 and 110 to 114) (244) (Fig. 2). During the early stage, matrix^{Gag} NLSs promote the nuclear localization of the PIC in nondividing cells (244). During the late stages of viral translation, the matrix^{Gag} NES is a dominant signal that counteracts the nuclear import activity of the matrix^{Gag} NLS to keep Gag in the

cytoplasm (242). Although the localization of HIV-1 Gag proteins in the nucleus has been proposed (214, 242), the nuclear trafficking ability of HIV-1 Gag remains debated (245). Overall, the binding of CRM1 to Rev and matrix^{Gag} plays a key role in the nuclear export of viral mRNA. Further investigation of Gag-mediated nuclear trafficking is still warranted.

Rev-CG1-Vpr Association

Human nucleoporin-like protein 1 (NLP-1 or CG1), which interacts with Rev and Vpr, is an important component of the nuclear pore complex (NPC) (246, 247). On the one hand, data from mammalian two-hybrid assays suggest a direct interaction between Rev and CG1 (246). The Rev-CG1 interaction, which requires the nuclear export signal of Rev (positions 75 to 83), plays a role in Rev-mediated nuclear export after viral transcription (246). On the other hand, GST pulldown assays, coimmunoprecipitation assays, and yeast two-hybrid assays demonstrate that CG1 physically interacts with Vpr (247). The Vpr-CG1 interaction enhances the docking of Vpr at the nuclear pore complex, leading to the accumulation of Vpr in the nuclear envelope (247). Although it is localized predominantly in the nuclear envelope, Vpr harboring two nuclear localization signals (positions 17 to 34 and 46 to 74) shuttles rapidly between the nuclear and cytoplasmic compartments (247–249). Regarding the interaction domains, alpha-helix regions of Vpr (positions 17 to 46 [247], L23, and K27 [248]) may interact with the N-terminal region of CG1 (positions 94 to 170 [247]). Overall, the binding of the human protein CG1 to HIV-1 Rev and Vpr plays an important role in HIV-1 nuclear export.

Tat-p300/SWI/SNF-Integrase Association

Although a direct interaction between Tat and integrase has not been reported, HIV Tat and integrase are colocalized in the nucleus (Fig. 3), permitting a possible association through nuclear proteins. The transcriptional coactivator and histone acetyltransferase p300 is known to regulate chromatin conformation and DNA transcription (250). Concrete evidence suggests that p300 physically interacts with HIV-1 Tat (251–254) and integrase (255, 256). On the one hand, the acetyltransferase p300 acetylates two lysine residues (K50 and K51) in HIV-1 Tat, resulting in an improvement of the Tat-mediated transcriptional activation of the HIV-1 promoter as well as an increased binding affinity of acetylated Tat for core histones (251, 253). In addition to K50 and K51, p300 also acetylates K28 in the activation domain of Tat to promote HIV-1 transcription (257). On the other hand, HIV-1 integrase is subject to posttranslational modifications by cellular cofactors (e.g., p300) (258, 259). During viral integration, p300 acetylates three lysine residues (K264, K266, and K273) in the C-terminal domain of viral integrase (255, 256). Although it remains debated (258), the p300-mediated acetylation of HIV-1 integrase might increase the binding affinity of integrase for nucleosomal DNA and promote the integrase strand transfer reaction (255). In addition to p300, other cellular cofactors (e.g., CREB and GCN5) are recruited by Tat to promote HIV-1 transactivation (260–263). Overall, p300 acts as an acetyltransferase to alter the activities of HIV-1 Tat and integrase.

The SWI/SNF (switch/sucrose nonfermenting) complexes are a family of ATP-dependent chromatin-remodeling complexes that utilize the energy of ATP hydrolysis to remodel the nucleosome in order to make the DNA accessible during transcription, replication, and DNA repair (264, 265). Human SWI/SNF complexes consist of multiple subunits, such as a single ATPase (either BRM or BRG1), three core subunits (BAF47 [also called INI1 or SNF5], BAF155, and BAF170), and several accessory subunits (e.g., β-actin) (264). Among these subunits, INI1 (266, 267), BRM (268), BRG1 (269), and β-actin (269) can physically interact with HIV-1 Tat, while INI1 binds to HIV-1 integrase (270–272). To activate viral transcription, Tat binds to the transactivation response (TAR) element, which is a 59-nucleotide stem-loop in viral RNA (29, 273). Subsequently, Tat recruits pTEFb (a structural complex with CDK9 and cyclin T1), SWI/SNF chromatin-remodeling complexes, and other cellular cofactors to the HIV-1 promoter (268, 274). In this process, p300 acts synergistically with INI1 and BRG1 to activate the HIV-1 promoter (269). As for the integrase-SWI/SNF interaction, the binding of the INI1 subunit of SWI/SNF to HIV-1 integrase not only promotes the efficient integration of viral DNA into stable nucleosomes (275, 276) but also enhances the packaging of INI1 (270). Overall, the meticulous associations between viral proteins (Tat and integrase) and cellular cofactors (p300 and SWI/SNF) in the nucleus play an important role in HIV-1 integration and transcription.

VIRAL ASSEMBLY AND BUDDING

As illustrated in Fig. 9, HIV genomic RNA, Vif, Nef, Vpr, Env, Gag/GagPol precursors, and host factors (e.g., TIP47 [tail-interacting protein of 47 kDa] and lipid) are assembled into nascent HIV virions, subsequently pinching off from the cell membrane (2, 30, 277). The presence of multiple HIV proteins in nascent virions thus establishes a basis for HIV pairwise protein interactions. The amounts of HIV-1 proteins per virion have been quantified in the literature.

For Gag and GagPol, an immature HIV-1 particle contains ~2,400 ± 700 copies of Gag precursors, quantified by electron tomography and scanning transmission electron microscopy (278). Dot blot assays also measured the ratio of Gag to GagPol to be 20:1 (279), corresponding to ~120 GagPol copies per virion.

For Env, cryo-electron microscopy tomography shows ~14 Env spikes per HIV-1 virion (280). Typically, each HIV virion contains 5 to 15 Env spikes (22).

For Vif, semiquantitative Western blot analyses estimated an average of 30 to 80 copies of Vif per virion (281). Although controversial results have been reported, it is generally agreed that there are <100 Vif copies per virion (43, 282, 283).

For Vpr, phosphorimage analyses estimated the molar ratio of Vpr to capsid to be ~1:7 (284). Findings from X-ray crystallographic analyses suggest that the HIV-1 capsid lattice consists of ~250 hexamers and exactly 12 pentamers of capsid (285), which correspond to ~1,560 capsid monomers per virion. Therefore, ~220 copies of Vpr might be encapsulated per virion.

For Nef, autoradiography and bioimager analyses estimated that ~5 to 10 copies of Nef are incorporated per virion (286).

Previous studies have reviewed the roles of Env trafficking and packaging (5, 287), HIV-1 genome packaging (288), membrane lipids (289), and cellular cofactors (290) in promoting viral budding. Here, we focus on HIV-1 pairwise protein interactions and associations during viral budding.

Matrix^{Gag}-GP41^{Env} Interaction

The GP41CT can physically interact with matrix^{Gag} (69–76). The matrix^{Gag}-GP41CT interaction takes part in multiple activities

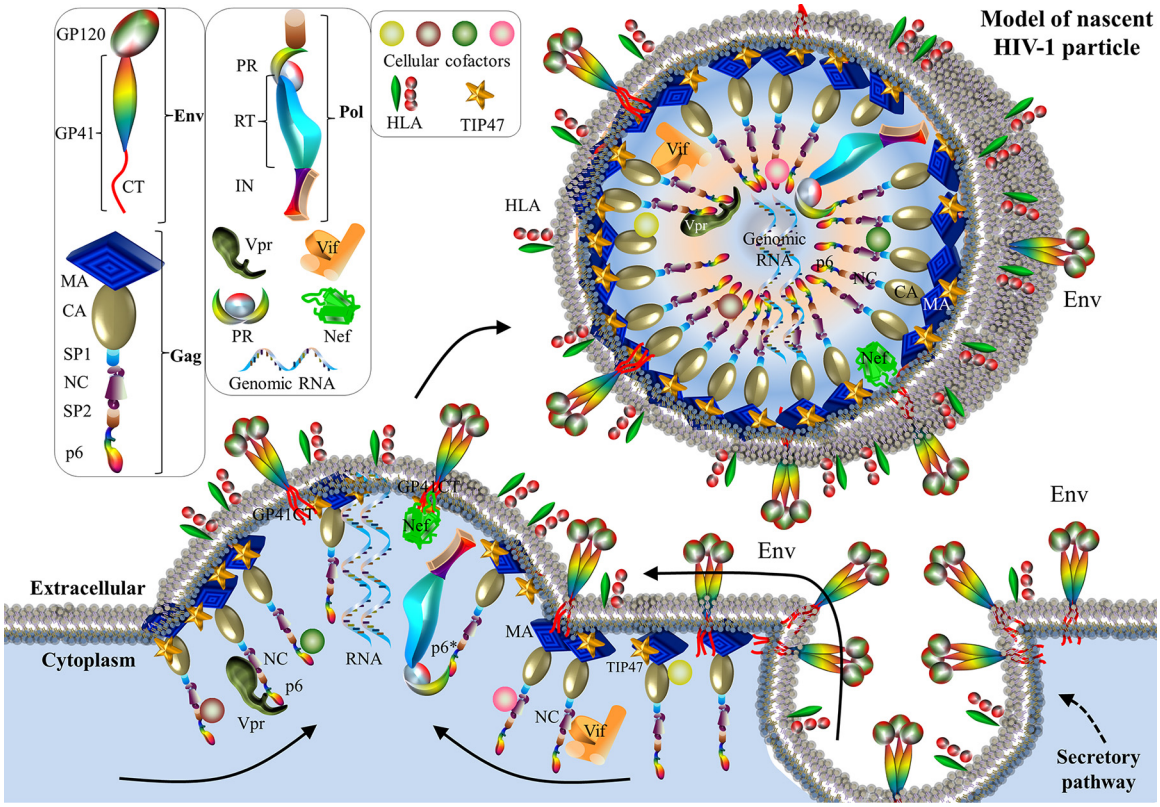


FIG 9 Schematic model of HIV-1 protein interactions during viral budding. Env trimers are exported to the extracellular membrane through a secretory pathway (617). Gag and GagPol are targeted to glycolipid-enriched membrane lipid rafts, where cholesterol, sphingolipids, and glycosylphosphatidylinositol-linked proteins are abundant (618). Several HIV protein interactions have been observed. The first interaction is the matrix^{Gag}-GP41^{Env} interaction. Mediated by the cellular cofactor TIP47, matrix^{Gag} interacts with GP41^{Env} for Env packaging into HIV particles (297). Another is the Vpr-Gag interaction. Vpr is incorporated into nascent virions through the binding of Vpr to NC^{Gag} and p6^{Gag} (315, 316, 324). A third interaction is the Vpx-p6^{Gag} interaction. HIV-2 Vpx binds to p6^{Gag} for Vpx packaging (333). A fourth interaction is the NC^{Gag}-Vif interaction. NC^{Gag} interacts with Vif for the packaging of Vif (300–303). A fifth interaction is the Env-Nef interaction. Nef enhances the packaging of Env (339, 341). On the viral membrane, ~50 to 63 HLA-II complexes are incorporated per virion (619). As a capsid-encoding virus (620), HIV is featured by its fullerene cone harboring ~250 hexamers and exactly 12 pentamers of HIV-1 capsid (285). Protein shapes do not indicate exact protein structures, nor are the protein sizes to scale.

during viral budding. (i) This interaction promotes Env packaging (76, 291). (ii) Although the entire matrix^{Gag} protein is dispensable for Env packaging (182), the matrix^{Gag}-GP41^{Env} interaction enhances the proper intracellular transport of Env glycoproteins in HIV-infected CD4^{+} T lymphocytes (292). (iii) Given that Gag and Env proteins are colocalized in the plasma membrane (293), the matrix^{Gag}-GP41CT^{Env} interaction permits the efficient association of Env glycoproteins anchored with lipid rafts on the extracellular membrane (294). (iv) Gag may determine the inhibition of Env internalization because in the absence of Gag, Env on the extracellular membrane is rapidly internalized through endocytosis (295). The presence of Gag decreases the rate of Env internalization by targeting the internalization motif in the HIV-1 GP41CT (295). (v) Matrix trimerization that builds a lattice capable of accommodating the GP41CT is crucial for Env packaging (296). (vi) Human proteins may exert an impact on the matrix^{Gag}-GP41^{Env} interaction. For instance, the cellular cofactor TIP47 interacts with both matrix^{Gag} and Env during viral budding (297). Because of this, the overexpression of TIP47 promotes Env packaging, whereas TIP47 depletion can abolish Env packaging (297).

Regarding the interaction domains, data from mutagenesis analyses suggest that amino acid substitutions (e.g., E16K, K29E, K31E, and E99V) in matrix^{Gag} can impair Env packaging (74, 75, 292, 298). Substitutions at serine positions (S9, S67, S72, and S77) in HIV-1 matrix^{Gag} dramatically reduce the phosphorylation of matrix^{Gag} and inhibit the binding of matrix^{Gag} to lipid rafts, thereby causing an impairment of Env packaging (299). Moreover, three matrix^{Gag} substitutions (L12E, L30E, and V34I) efficiently block Env packaging (71, 76), whereas Y712C/F substitutions in the GP41CT compensate for the impaired infectivity of virions with the three matrix^{Gag} substitutions described above (70). Other studies have also confirmed that L12 and V34 in matrix^{Gag} play a critical role in Env packaging (69, 76).

NC^{Gag}-Vif Interaction

Vif physically interacts with nucleocapsid in the Gag precursor (NC^{Gag}), an interaction identified by coimmunoprecipitation assays, GST pulldown assays, phage display assays, and mammalian two-hybrid assays (300–303). This interaction works in three aspects. (i) Vif packaging into nascent HIV-1 particles not only requires the binding of Vif to two zinc finger domains in NC^{Gag} but also depends on the interaction between Vif and viral genomic RNA (300). In the absence of Vif, NC^{Gag} is less stably packaged

into the HIV-1 core (304). Moreover, Vif and NC^{Gag} share common binding sites on $tRNA^{Lys3}$, a cellular primer which is incorporated into HIV-1 particles (305). (ii) Despite a small amount of Vif being imported into HIV-1 particles (306), the Vif-NC^{Gag} interaction promotes the stability of HIV-1 core and prevents the premature degradation or the disassembly of nucleoprotein complexes (304). Vif negatively regulates the NC-assisted maturation of the viral RNA dimer in nucleoprotein complexes, which in turn prevents the premature initiation of reverse transcription (133). (iii) Vif selectively inhibits protease-mediated proteolytic cleavage between NC^{Gag} and $SP1^{Gag}$ (307). In order to keep a low level of expression of Vif that inhibits protease activity, most newly synthesized Vif proteins are rapidly degraded by cellular proteases (307). (iv) Vif induces the degradation of the human cytidine deaminase APOBEC3G via the ubiquitin-proteasome pathway, thereby preventing the packaging of APOBEC3G into nascent HIV particles (308, 309). When this Vif-mediated degradation is impaired under certain circumstances, APOBEC3 proteins are efficiently incorporated into nascent HIV-1 particles through their interactions with NC^{Gag} or $matrix^{Gag}$ (310).

Regarding the interaction domains, two zinc finger domains of nucleocapsid are essential for Vif packaging (300). Early studies suggested that nucleocapsid may interact with the motif at the C terminus of Vif (positions 171 to 192) (303) or Vif domains (positions 68 to 81, 89 to 100, 162 to 173, and 177 to 189) (301). Subsequent studies reanalyzed the role of the Vif C terminus and refuted its interaction with NC^{Gag} (302, 311). Instead, the N-terminal domain (positions 1 to 22) and the central domain (positions 70 to 100) of Vif might interact with NC^{Gag} (302).

NC^{Gag}-Vpr Interaction

HIV-1 Vpr can interact with nucleocapsid (NC^{Gag}) in Gag precursors, an interaction detected by two-hybrid assays, far-Western blot assays, Vpr binding assays, and competition experiments using agarose bead-immobilized avidin (312–314). During the early assembly of Gag, Vpr is encapsulated into nascent viral particles via its interaction with Gag (312–316). Vpr multimerization is crucial for the Gag-Vpr interaction (315). On the one hand, NC^{Gag} cooperates with $p6^{Gag}$ to promote Vpr packaging (313), although NC^{Gag} is dispensable for Vpr packaging (317). On the other hand, Vpr enhances the transcription of unspliced *gag* transcripts expressed from unintegrated viral DNA (318). In contrast to the Vpr-NC^{Gag} interaction in HIV, Vpr and Vpx in SIVsm do not bind to NC^{Gag}, suggesting a distinct difference between HIV and SIVsm proteins (314). In addition, the NC-Vpr structural complex can activate phosphatase $2A_0$, a cellular protein that inhibits the transition of the cell cycle (319).

Regarding the interaction domains, the C-terminal helix domain of Vpr (positions 70 to 80) may interact with NC^{Gag} (313). An early study proposed that two zinc finger domains of NC^{Gag} may interact with Vpr (313), but this result was refuted by a subsequent study (314). Future studies are needed to verify their interaction domains.

$p6^{Gag}$-Vpr Interaction

Vpr can be incorporated into nascent viral particles via its interaction with the p6 domain ($p6^{Gag}$) in Gag precursors (314, 320–330). This interaction has been identified by using yeast two-hybrid assays, immunoprecipitation assays, maltose-binding protein pulldown assays, nuclear magnetic resonance, fluorescence lifetime imaging microscopy, and plasmon waveguide resonance spectroscopy techniques (314, 315, 322, 328). The binding affinity between Vpr and $p6^{Gag}$ is increased under the environment of lipid bilayer membranes (328). Notably, $p6^{Gag}$ cooperates with NC^{Gag} to promote Vpr packaging (313).

Regarding the interaction domains, the $F^{15}RFG^{18}$ motif (329) and the leucine-rich motif ($L^{41}XXLF^{45}$) of $p6^{Gag}$ (314, 320, 325–327, 330) may interact with the N-terminal domain of Vpr (positions 18 to 34 [322] and 1 to 71 [327]). The phosphorylation of position S487 in $p6^{Gag}$ promotes the Gag-Vpr interaction and enhances Vpr packaging (331).

$p6^{Gag}$-Vpx Interaction

HIV-2 and simian immunodeficiency virus (SIV) Vpx proteins can interact with $p6^{Gag}$ according to coimmunoprecipitation assays, GST pulldown assays, yeast two-hybrid assays, and *in vitro* binding assays (314, 320, 332, 333). The Vpx-$p6^{Gag}$ interaction drives Vpx packaging into HIV-2 and SIV particles (314). In contrast to the HIV-1 Vpr-NC^{Gag} interaction, SIV Vpx does not interact with NC^{Gag} (314). In nondividing cells, the conserved domain of Vpx (positions 60 to 85) in HIV-2 and SIV is essential for PIC nuclear import (334, 335). Regarding the interaction domains, the leucine-containing motif $D^{17}XAXXLL^{23}$ in $p6^{Gag}$ (320) may bind to SIVmac Vpx (320) and HIV-2 Vpx (positions 73 to 89) (332, 333).

$GP41^{Env}$-Nef Interaction

HIV-1 binding assays suggest that Nef can physically interact with the GP41CT (336). This interaction potentially offers a distinct feature for Nef to protect HIV-1 virions from potent neutralizing monoclonal antibodies (e.g., 2F5 and 4E10) that target the GP41CT (337). As an accessory protein with multiple activities, Nef not only is involved in CD4 downregulation (338, 339) but also enhances viral entry involving CD4 and chemokine receptors (286, 340). In the former case, Nef downregulates CD4 to prevent the aggregation of Env-CD4 complexes in the endoplasmic reticulum (17, 341, 342), thereby inhibiting CD4 packaging into HIV-1 particles of either CCR5- or CXCR4-tropic strains (343). HIV-1 Nef also collaborates with Env to activate plasmacytoid dendritic cells for the production of interferon alpha (IFN-α) based on CD4-dependent mechanisms (338). Overall, Nef counteracts the inactivation of trimeric Env spikes to promote Env packaging (344), although Nef is dispensable for viral infection (345).

Regarding the interaction domains, data from mutagenesis analyses suggest that the C-terminal domain of Nef (positions 181 to 210) may interact with the C-terminal dileucine motif (positions 712 to 715) of the GP41CT (336). Deletion of the GP41CT abrogates the Nef-induced enhancement of viral infectivity in HIV-infected $CD4^+$ T lymphocytes (336). Moreover, the tyrosine-based sorting motif ($Y^{712}XXL^{715}$) in the GP41CT is required for the efficient intracellular trafficking of Env glycoproteins (346). Future studies are still needed to verify the reproducibility of the GP41CT-Nef interaction.

Gag-RT Interaction

Findings from coimmunoprecipitation assays and Western blot analyses suggest that HIV-1 RT interacts with Gag for RT packaging (347, 348). HIV-1 $matrix^{Gag}$ and $p6^{Gag}$ in Gag might interact with RT, covering the thumb domain of RT (347). However, the

occurrence of HIV-1 RT packaging is likely limited, because it is rare for RT encoded by GagPol to be cleaved by viral protease and to become mature before viral budding. Precise interaction domains in RT and Gag remain unclear. For future studies, independent experiments are still needed to verify the reproducibility of the Gag-RT interaction.

Matrix^{Gag}-RNA-NC^{Gag} Association

To our knowledge, a direct interaction between matrix and nucleocapsid has not been reported, despite the finding that the N-terminal domain of matrix^{Gag} in Gag might fold back onto the C-terminal domain of NC^{Gag} for the regulation of Gag assembly (349). During viral budding, viral genomic RNA interacts with two Gag domains: matrix^{Gag} (350–355) and NC^{Gag} (351, 353, 356–360). The basic residues of HIV-1 NC^{Gag} and the N-terminal region of matrix^{Gag} ($K^{26}KQYK^{30}$) are required for the packaging of viral genomic RNA (351, 352, 358). Before viral budding, and after viral budding and maturation, NC^{Gag} preferentially binds to the psi and Rev response elements in the viral genomic RNA, while NC^{Gag} binds to many sites on the HIV-1 genome (353). In addition to viral RNA, many host proteins (e.g., importin-α/β) also interact with matrix^{Gag} and NC^{Gag} for Gag packaging and intracellular trafficking (361).

The matrix^{Gag}-RNA-NC^{Gag} association allows multiple activities. (i) This association is critical for genomic RNA packaging (356). From a broad perspective, viral matrix^{Gag} generally contributes to RNA binding and genomic RNA packaging in deltaretroviruses (e.g., bovine leukemia virus) (362). (ii) The binding of Gag to genomic RNA contributes to Gag multimerization (351, 357, 363). (iii) Before the delivery of Gag to appropriate budding sites, the binding of viral RNA to matrix^{Gag} protects matrix^{Gag} from its association with inadequate cellular membranes (350, 355). Thiadiazolanes that target the matrix^{Gag}-RNA interaction can inhibit HIV-1 replication (354). (iv) To promote virus budding, viral RNA can downregulate Gag membrane binding (364). In the absence of phosphatidylinositol 4,5-bisphosphate [$PI(4,5)P_2$], viral RNA interacts with matrix^{Gag} to abolish the binding of Gag to liposomes (364). (v) Matrix^{Gag} inhibits the annealing of primer tRNA^{Lys3} onto viral genomic RNA, whereas NC^{Gag} is essential for tRNA^{Lys3} annealing (365). Matrix^{Gag} exclusively interacts with cellular tRNAs (e.g., tRNA^{Lys3}) in the cytosol, thereby regulating Gag binding to cell membranes (353). Overall, the matrix^{Gag}-RNA-NC^{Gag} association plays a critical role in viral budding.

Capsid^{Gag}-LysRS-Vpr Association

Recognized as a conserved cellular enzyme, lysyl-tRNA synthetase (LysRS) takes part in protein synthesis and circulates in multiple compartments (the nucleus, mitochondria, and plasma membrane) for transcriptional regulation, cytokine-like signaling, and the transport of proteins to the cell membrane (366). Although a direct interaction between capsid and Vpr has not been reported to our knowledge, LysRS physically interacts with both HIV-1 Vpr (159) and capsid^{Gag} (367–369). On the one hand, HIV-1 Vpr interacts with LysRS to inhibit the LysRS-catalyzed aminoacylation of tRNA^{Lys3}, although the accessory protein Vpr is dispensable for viral production (159). During viral budding, tRNA^{Lys3} is incorporated into HIV-1 particles (367, 370, 371). On the other hand, newly synthesized LysRS binds rapidly to capsid^{Gag} on the plasma membrane during the course of viral budding, thereby avoiding the localization of LysRS to other cellular compartments (367). This process permits the incorporation of ~25 LysRS molecules per HIV-1 particle (372).

Regarding the interaction domains, the C-terminal domain of capsid (positions 177 to 231) may interact with amino acid positions 208 to 259 within the dimerization helix of LysRS (369, 373). The homodimerization of Gag and LysRS indeed contributes to the capsid^{Gag}-LysRS interaction (368). LysRS interacts with the N-terminal domain of HIV-1 Vpr (positions 1 to 39) (159). Overall, LysRS binds to capsid^{Gag} for its packaging and interacts with Vpr to prevent the LysRS-mediated aminoacylation of tRNA^{Lys3}.

Gag-AIP1-Nef

As an accessory protein known for its multiple activities, Nef not only increases the total amount of Gag proteins localized at the plasma membrane but also enhances cell-to-cell viral infection in primary $CD4^+$ lymphocytes (374). Although a direct interaction between Gag and Nef has not been reported to our knowledge, both NC^{Gag} and Nef bind to AIP1 for efficient viral budding (375–378). AIP1 (apoptosis-linked gene 2 [ALG2]-interacting protein 1) (also known as Alix or PDCD6IP) is associated with the endosomal sorting complex required for transport (ESCRT) machinery (377). The ESCRT machinery is known for promoting cargo sorting and multivesicular body biogenesis (379). On the one hand, GST pulldown assays demonstrate the physical interaction between NC^{Gag} and the Bro1 domain of AIP1 (378). On the other hand, data from GST pulldown and coimmunoprecipitation assays suggest that Nef physically binds to AIP1 (375, 377). The Nef-AIP1 interaction not only promotes the proliferation of multivesicular bodies (375) but also facilitates CD4 degradation through lysosomal pathways (377). HIV-1 Nef and glycosylated Gag cooperatively downregulate two transmembrane proteins, serine incorporator 3 (SERINC3) and SERINC5, from the cell surface to prevent their packaging, consequently counteracting their antiviral activity (380).

Regarding interaction domains, the Brol1 and V domains of AIP1 interact with Nef (377). The first 202 positions in the Brol1 domain of AIP1 bind to the zinc finger and N-terminal domains of NC^{Gag} (376, 378, 381), particularly key positions such as R3, R7, R10, K11, K14, K20, and R26 (382). Data from crystallization analyses also suggest that amino acid position F105 at the unique extended loop of AIP1 is crucial for HIV-1 budding (383). Moreover, the $Y^{135}PLT^{138}$ motif in Nef may interact with AIP1 (375).

NC^{Gag}-Tsg101/AIP1-P6^{Gag} Association

Two cellular proteins, Tsg101 and AIP1 (also termed Alix), are important components of the ESCRT machinery, which initiates protein sorting into late endosomes (379, 384). During the early stage of viral budding, the Gag protein in its ubiquitinated form recruits AIP1 and Tsg101/ESCRT-I to initiate ESCRT-mediated assembly (385). Thereafter, the downstream ESCRT-III and VPS4 factors are recruited to complete viral budding (386). Experimental evidence suggests that HIV-1 NC^{Gag} physically interacts with AIP1 (378) and Tsg101 (387). In addition, HIV-1 p6^{Gag} interacts with AIP1 (386, 388, 389) and Tsg101 (390). During viral budding, AIP1 is packaged into viral particles through the interaction between the Bro1 domain of AIP1 and the zinc finger domains of NC^{Gag} (378). Moreover, the binding of Gag to the ESCRT machinery is vital for virus scission from the extracellular membrane of HIV-infected cells (382). Notably, the NC^{Gag}-AIP1 interaction re-

quires the involvement of RNA (376) and the cellular protein galectin-3 (391). In the former case, viral RNA bridges the interaction between NC^{Gag} and AIP1 (376). In the latter case, galectin-3 interacts with AIP1 to promote the AIP1-$p6^{Gag}$ interaction (391).

Regarding the interaction domains, the Bro1 and V domains of AIP1 can bind to NC^{Gag} and $p6^{Gag}$, respectively (376, 378). The N-terminal basic residues and zinc finger domains of NC^{Gag} interact with AIP1 (381). Two motifs, $P^{7}TAP^{10}$ and $L^{35}YPX_nL$, in $p6^{Gag}$ interact with the cellular proteins Tsg101 and AIP1, respectively (381). Moreover, the C-terminal proline-rich domain of AIP1 (positions 391 to 510) may interact with $p6^{Gag}$ (388), while the N-terminal domain of Tsg101 binds to the $P^{7}TAPP^{11}$ motif in $p6^{Gag}$ (390). Overall, NC^{Gag} and $p6^{Gag}$ in Gag cooperatively interact with Tsg101 and AIP1 to recruit the ESCRT machinery for viral budding.

Vif-APOBEC3G-Integrase Association

Although a direct interaction between HIV-1 integrase and Vif has not been reported, integrase and Vif interact with apolipoprotein B mRNA-editing catalytic polypeptide-like 3G (APOBEC3G or A3G) (33–43). APOBEC3 proteins from the human APOBEC3 family of DNA cytosine deaminases are known as anti-HIV cellular proteins that impair viral DNA synthesis and integration by introducing G-to-A hypermutation to the viral genome (43, 392–394). APOBEC3G physically interacts with HIV-1 integrase to prohibit the formation of proviral DNA (33). To counteract this restriction, Vif physically interacts with APOBEC3G for the degradation of APOBEC3G (35, 36, 39–42, 395). HIV-1 Vif in complex with cellular proteins (e.g., Cul5, Rbx1, and elongins B and C) induces the ubiquitination and proteasomal degradation of APOBEC3G in the cytoplasm (38). Moreover, Vif interacts with the transcription cofactor CBF-β to enhance the degradation of APOBEC3G (396). In studies of four subspecies of African green monkey, it has been shown that APOBEC3G is adaptively diversifying within hosts because of the antagonism-driven coevolution between Vif and APOBEC3G (397). In addition to APOBEC3G, Vif binds to other proteins in the human APOBEC3 family (e.g., APOBEC3C [398, 399] and APOBEC3F [43, 400]). Alanine-scanning analyses revealed six Vif residues (D14, R15, M16, W79, D172, and W174) in three conserved motifs that are essential for the degradation of APOBEC3C and APOBEC3F (401).

Interaction domains of integrase-APOBEC3G and APOBEC3G-Vif interactions have been investigated by extensive studies. In the former case, the C-terminal domain of HIV-1 integrase (positions 213 to 288) may interact with the link domain of APOBEC3G (positions 104 to 156) (33). In the latter case, HIV-1 Vif binds to the N-terminal domain of APOBEC3G within the α1-β1, β2-α2, and β4-α4 loop regions (395). The $P^{161}PLP^{164}$ motif of Vif is essential because mutations at this motif disrupt the Vif-APOBEC3G interaction, triggering the escape of APOBEC3G, which allows APOBEC3G packaging into nascent HIV particles (36). In summary, many Vif motifs have been found to interact with APOBEC3G, such as $D^{14}RMR^{17}$ (39), $W^{21}xSLVK^{26}$ (402), $Y^{40}RHHY^{44}$ (39), $V^{55}xIPLx_{4-5}Lx\phi x_2YWxL^{72}$ (403), $Y^{69}xxL^{72}$ (404), $L^{81}GxGxxIxW^{89}$ (41), $P^{161}PLP^{164}$ (36, 405), $E^{171}DRW^{174}$ (41), and $T(Q/D/E)x_5ADx_2(I/L)$, located between positions 96 and 107 (42). Additional studies are still needed to investigate whether these Vif motifs vary due to different protein interaction interfaces or due to different experimental settings.

Vif-MAPK/HCK-Nef Association

MAPK is a serine/threonine/tyrosine-selective protein kinase that phosphorylates multiple HIV-1 proteins, such as matrix, Vif, Tat, Rev, and Nef (406). Specifically, MAPK phosphorylates HIV-1 Vif positions T96 and S165 to downregulate viral replication (407). To counteract this defect, the proline-rich repeat region of Nef (positions 69 to 83) physically interacts with MAPK (408). This interaction allows HIV-1 Nef to inhibit the kinase activity of MAPK (408). In fact, Nef not only alters the activity of the MAPK pathway for T-cell receptor stimulation in primary CD4 T cells (409) but also induces many transcription factors (e.g., activator protein 1) (410). In astrocytes, MAPK and hematopoietic cell kinase (HCK) signaling pathways take part in the production of Nef-induced interleukin-6 (IL-6), interleukin-8 (IL-8), and chemokine (C-C motif) ligand 5 (CCL5) (411, 412).

As a member of the Src family of tyrosine kinases, HCK plays a role in the innate immune response and many signaling pathways (413, 414). HCK physically interacts with HIV-1 Vif (415, 416) and Nef (417–422). Specifically, the SH3 domain of HCK binds to the proline-rich motif $P^{72}QVP^{75}$ in the N-terminal anchor domain of Nef (422, 423) as well as the $P^{161}PLP^{164}$ motif in HIV-1 Vif (415). The SH2 and SH3 domains of HCK physically interact with the $P^{72}xxP^{75}$ motif of HIV-1 Nef to stabilize the functional conformation of the Nef dimer (421). Multiple functions of the Vif-HCK-Nef association have been characterized. (i) Vif interacts with HCK to counteract the HCK-mediated inhibition of viral release (416). (ii) HIV-1 Nef can selectively activate HCK (418). This activation can inhibit the functions of macrophage colony-stimulating factor (M-CSF) receptor in monocytes and macrophages (424). The direct interaction between Nef and HCK induces the activation of HCK, which is indispensable for the downregulation of the M-CSF receptor Fms accumulated as an immature protein at the Golgi apparatus (420). (iii) The Nef-HCK interaction promotes viral growth of Nef-positive (Nef^+) viruses but does not alter CD4 downregulation (425). (iv) HCK is involved in the Nef-mediated downregulation of CD1 expression in dendritic cells (426). In addition to HCK, other Src family members (e.g., Lyn and c-Src) interact with Nef (418).

Overall, Nef activates the HCK pathway to downregulate cell surface receptors (419, 420), but Vif counteracts the HCK-mediated inhibition of viral release. Moreover, MAPK phosphorylates Vif, whereas Nef inhibits the kinase activity of MAPK.

Vpu-CD4-$GP120^{Env}$ Association

As a key receptor on cell membranes, CD4 is known to physically interact with GP120 (427–432) and Vpu (14, 19, 433). Due to the flexible nature of HIV-1 Env, the closed and open conformations of Env drive the dynamic binding of Env to CD4, coreceptors, and antibodies (56, 434). To promote viral entry, GP120 interacts with CD4 to trigger profound dynamic structural rearrangements and to induce the aggregation of CD4 and coreceptors (e.g., CCR5) (427, 435–439). During viral budding, this aggregation, however, prohibits the packaging of Env into viral particles (12–17). As of today, a large number of antibodies (e.g., VRC01) that target CD4-binding sites in GP120 have been demonstrated to have a broad and potent activity of neutralization (440–446). Many antiviral agents (e.g., NBD556) against the GP120-CD4 interaction are under investigation (447–449).

As an accessory protein with high sequence variability, HIV-1

Vpu not only enhances viral budding but also induces CD4 degradation (14, 15, 450). HIV-1 Vpu is colocalized with Env and Gag in the *trans*-Golgi network (451). The binding of HIV-1 Vpu to newly synthesized CD4 causes the retention of CD4 in the ER and, subsequently, the delivery of CD4 to the ER-associated degradation pathway (12–19). The rapid downregulation of CD4 reduces the amount of Env-CD4 structural complexes in the ER, thus promoting the transport of Env to the Golgi apparatus, where oligosaccharide modifications take place (12, 13, 19, 452). The Vpu-CD4 interaction requires the phosphorylated Vpu monomer as the active structure, because monomeric but not multimeric Vpu acts on CD4 downregulation (19), and phosphorylated but not nonphosphorylated Vpu triggers CD4 degradation (453). Many cellular proteins, such as βTrCP, also take part in the Vpu-dependent degradation of CD4 (15, 18). Overall, HIV-1 Vpu induces CD4 degradation to promote Env packaging.

Vpu-Tetherin/CD4-Nef Association

Although a direct interaction between HIV-1 Vpu and Nef is yet to be explored, Vpu and Nef share common activities to downregulate various host proteins (e.g., tetherin, CD4, and CD62L) (341, 454, 455). It has been shown that human tetherin (also termed BST2) interacts with Vpu (456–463) and Nef (464). Meanwhile, CD4 physically binds to Vpu (14, 19, 433) and Nef (465). To explore possible associations between HIV-1 Vpu and Nef, we focus on the downregulation of tetherin and CD4 induced by Vpu and Nef.

Human tetherin is known for its antiviral activity that restricts viral budding, whereas this mechanism is antagonized by HIV-1 Vpu and Nef, resulting in the promotion of viral budding (457, 466–468). Although the exact mechanisms of Vpu action remain debated (469), the binding of HIV-1 Vpu to tetherin leads to the capture of tetherin (459) and subsequently to the downregulation of tetherin via lysosomal and/or proteasomal degradation (459, 461). Different aspects of this mechanism have been reported. (i) HIV-1 Vpu hijacks trafficking pathways of the clathrin adaptor protein complex 1 (AP1) and adaptor protein complex 2 (AP2) to induce postendocytic membrane trafficking events that remove tetherin from the cell membrane (456, 458). (ii) Despite the fact that tetherin enhances the susceptibility of HIV-infected cells to antibodies, HIV-1 Vpu and Nef antagonize tetherin to protect HIV-infected cells from antibody-dependent cell-mediated cytotoxicity, a type of human immune response where virus-specific antibodies activate the killing of HIV-infected cells (470–472). (iii) Among four HIV-1 groups (groups M, N, O, and P), only HIV-1 group M encodes Vpu that robustly counteracts human tetherin (473). Interestingly, the Nef-mediated antagonism of human tetherin is conceived to have evolved before the spread of HIV-1 group O (474).

HIV-1 Vpu and Nef act synergistically to counteract CD4 expression on the HIV-infected cell membrane that deleteriously blocks Env packaging into nascent HIV particles (341). On the one hand, HIV-1 Vpu determines CD4 downregulation by capturing newly synthesized CD4 in the ER, and the ER-retained CD4 is subsequently redistributed to the ER-associated degradation pathway (19). On the other hand, HIV-1 Nef in complex with AP2 interacts with the cytosolic tail of cell surface CD4, causing the internalization of cell surface CD4 to endosomes (475, 476). HIV-1 Nef also mediates the postendocytic targeting of internalized CD4 from the endosomes to the multivesicular body pathway, leading to the eventual degradation of CD4 in lysosomes (477). In addition to CD4, HIV-1 Vpu and Nef downregulate a broad spectrum of more than 32 cell surface receptors (454). Significant downregulation has been observed in the tetraspanin protein family harboring various cellular proteins (e.g., CD9, CD53, CD63, CD81, and CD82) that take part in membrane-based processes (454, 478). As another example, HIV-1 Nef and Vpu inhibit the adhesion and the signaling of L-selection (CD62L) to keep HIV-infected cells away from lymph nodes, a mechanism for HIV-1 to escape from host immune surveillance (455). In the presence of HIV-1 Vpu, Nef acts synergistically to downregulate PVR, a ligand that activates the receptor CD226 in natural killer cells and $CD8^+$ T cells against HIV-1 infections (479). Overall, HIV-1 Vpu and Nef can downregulate various cellular proteins to enhance viral budding.

Vpu-CK2-Rev Association

Casein kinase 2 (CK2) is a ubiquitous serine/threonine-selective protein kinase in all eukaryotes (480, 481). CK2 not only phosphorylates serine residues S52 and S56 at the cytoplasmic domain of HIV-1 Vpu (453, 482) but also phosphorylates HIV-1 Rev serine residues S5 and S8 to downregulate viral production (483, 484). In the former case, CK2-catalyzed phosphorylation is critical for Vpu-mediated CD4 degradation (453, 482). In the latter case, the regulatory beta subunit of CK2 binds to the N-terminal domain of Rev via electrostatic and hydrophobic interactions (483, 485). HIV-1 Rev harboring its arginine-rich domain (positions 35 to 50) activates CK2 to promote viral replication (484). Overall, the Vpu-CK2-Rev association makes it possible for Rev to trigger the activity of CK2, which phosphorylates HIV-1 Vpu for CD4 degradation.

Vpu-UBP-Matrix^{Gag} Association

Although a direct interaction between HIV-1 Vpu and Gag has not been reported, a Vpu-binding protein (UBP) has been identified to mediate the functional association between HIV-1 Vpu and matrix^{Gag} in Gag (486–489). HIV-1 Vpu can redistribute UBP and Gag to the intracellular membrane of HIV-1-infected cells (488). Newly synthesized Gag is initially transported to the extracellular membrane, where the endocytic uptake of Gag is dismissed in the presence of HIV-1 Vpu (490). In contrast, the absence of HIV-1 Vpu causes a significant amount of Gag to be redistributed to internal membranes for endocytosis (490). Interestingly, Vpu start codon mutants can rescue the impaired viral infectivity of a matrix mutant with the amino acid substitution L30E (491). Overall, HIV-1 Vpu collaborates with UBP to inhibit the endosomal accumulation of Gag in late endosomes, an inhibition process that enhances viral budding (492–494).

VIRAL MATURATION

After viral budding, immature HIV virions undergo maturation processes during which Gag and GagPol precursors are cleaved into mature proteins based on protease-mediated proteolytic processing (Fig. 10). Information about HIV maturation, core morphology, and Gag protein structures has been reviewed elsewhere (1, 2, 30, 31). Here, we focus on HIV-1 pairwise protein interactions that take place during viral maturation.

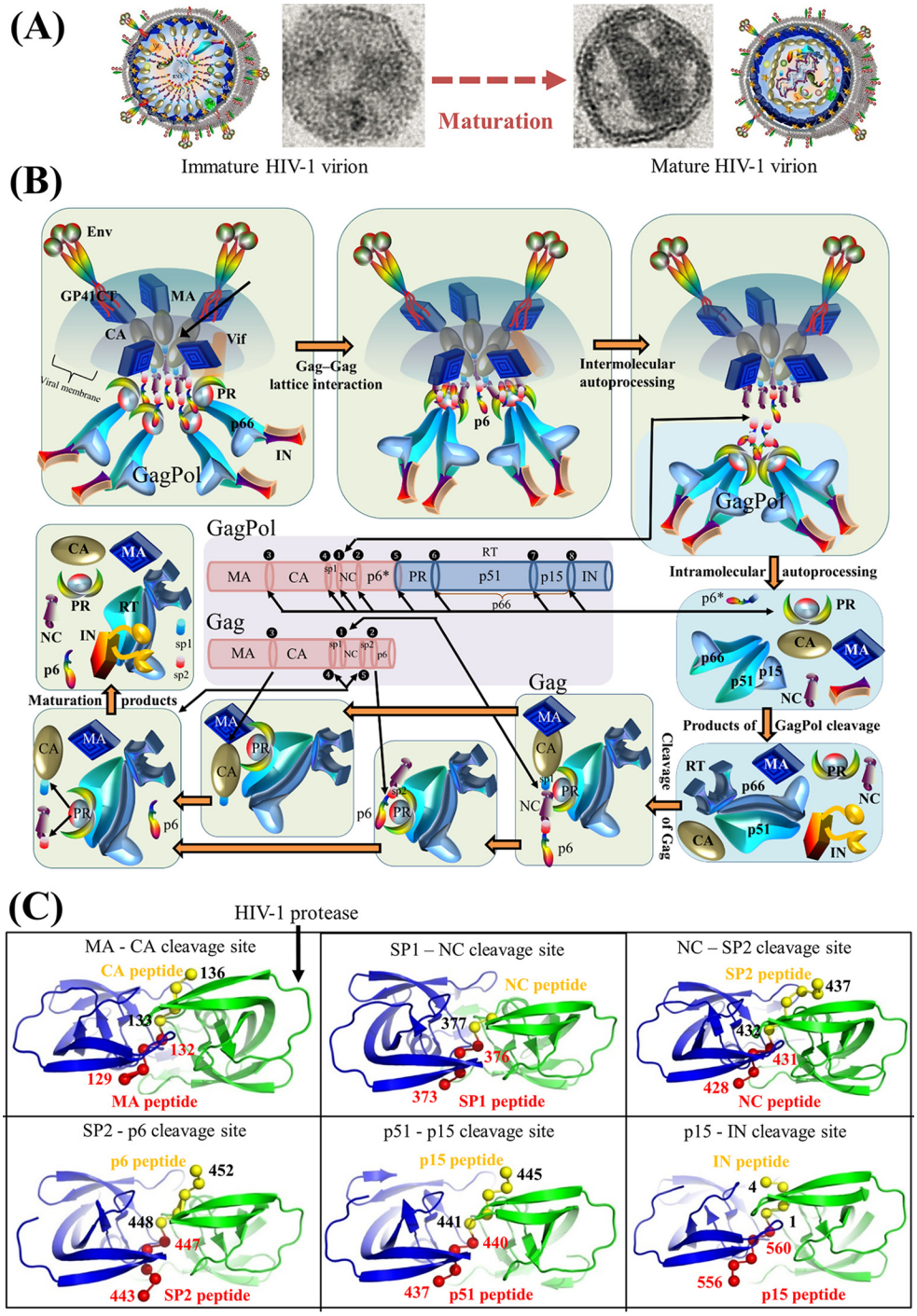


FIG 10 HIV-1 morphology, schematic model of protease-mediated proteolytic processing, and protease-substrate structural complexes. (A) Morphology of HIV-1 immature and mature particles during viral maturation. Schematic models of HIV-1 immature and mature particles are also illustrated. Protease-mediated proteolytic processing triggers the maturation of viral proteins inside HIV particles, resulting in significant changes of virion morphology. Electron micrograph images of HIV virions were obtained from the Centers for Disease Control and Prevention (CDC/Maureen Metcalfe and Tom Hodge; http://phil.cdc.gov/phil/details_linked.asp?pid=13472). (B) Protease-mediated proteolytic processing on Gag and GagPol precursors during viral maturation. Five steps have been recorded. First, GagPol dimerization within two GagPol precursors induces the activation of protease dimers with low enzymatic activity (507). Gag and GagPol dimerization occurs before their anchoring to the cell membrane, while GagPol is efficiently packaged into nascent viral particles through its interaction with Gag (621). The transframe region p6* in GagPol prevents premature protease-mediated proteolytic processing (508). The second step is protease-mediated intramolecular (*cis*) autoprocessing. The connection link between SP1 and nucleocapsid is cleaved by protease within the same GagPol dimer (622). The third step is protease-mediated intermolecular (*trans*) autoprocessing. HIV protease in one GagPol dimer further cleaves multiple cleavage sites (NC-p6*, p6*-protease, MA-CA, CA-SP1, protease-p51, protease-p66, p66-integrase, p51-p15, and p15-integrase) in another GagPol dimer (593, 623). After GagPol cleavage, HIV protease, integrase, RT, capsid, and nucleocapsid are folded properly. The fourth step is protease-mediated proteolytic processing of Gag. Mature viral protease cleaves Gag precursors in a specific order: SP1-NC, SP2-p6, MA-CA, CA-SP1, and NC-SP2 (31). This process releases MA, CA, SP1, NC, SP2, and p6 from Gag precursors. In the fifth step, during the maturation of HIV-1 proteins, a series of conformation rearrangements turns immature particles into mature particles (624). Note that protein shapes do not represent the exact protein structures, nor are the protein sizes to scale. (C) HIV-1 protease structures crystallized with 6 substrate peptides derived from Gag and GagPol cleavage sites. Upstream and downstream amino acids of Gag cleavage sites are shown in red and yellow, respectively. See Table S1 in the supplemental material for PDB accession numbers.

Protease-Gag/GagPol Interaction

HIV protease cleaves its substrates (Gag and GagPol) at specific cleavage sites (495–497), a mechanism called protease-mediated proteolytic processing (Fig. 10). This important mechanism allows the maturation of HIV structural proteins (matrix, capsid, nucleocapsid, and p6) and enzymatic proteins (protease, RT, and integrase), thereby transforming immature virions into mature virions with the full capacity for new viral infections (498). As of today, it remains a challenge to resolve the protein structures of full-length Gag and protease in order to identify exact interaction positions in Gag that bind to viral protease. Although the full-length structure of Gag in complex with viral protease has not been resolved, the tertiary structures of protease in complex with peptides near the Gag cleavage sites reveal that protease or Gag substitutions contribute to weak binding (499), low interaction energies (500), and reduced van der Waals contacts (501). Recent findings also suggest that amino acid substitutions in Gag induce significant resistance to protease inhibitors (PIs), causing treatment failure in patients receiving PI-based therapies (502–505). In the presence of protease drug-resistant mutations, cleavage site mutations in Gag and GagPol compensate for impaired protease-mediated cleavages (497, 505). This novel mechanism of HIV drug resistance is discussed below.

GagPol precursors harbor the transframe region p6* at the neighboring region of protease (Fig. 10). At the initial stage of viral maturation, the autoprocessing of HIV protease at the GagPol dimer produces mature viral enzymes (506, 507). As demonstrated by kinetic analyses and cross-linking experiments, p6* inhibits HIV-1 protease activity via a direct interaction (508–510). Multiple activities of p6* have also been reported. (i) p6* prevents protease activities until viral assembly is completed, therefore counteracting premature enzymatic processing (510). In line with this evidence, a complete deletion of p6* enhances protease-mediated proteolytic processing (511). (ii) Cleavages on incorrect p6* positions may significantly reduce the maturation of viral particles, highlighting the importance of accurate cleavages at p6* for protease activation (512). (iii) Although GagPol precursors lacking the p6* region could be incorporated into HIV-1 particles, p6* exerts negative regulation on protease dimerization, and it is indispensable for protease-mediated maturation (513).

Although interaction domains are yet to be explored, non-active-site residues and the C-terminal domain of p6* may regulate embedded protease functions by modulating protease conformations (514). The hydrophilic tripeptides (E^4DL^6 and E^4DF^6) at the N-terminal domain (509) and $S^{65}FNF^{68}$ at the C-terminal domain (508) of p6* may block the substrate-binding cleft of HIV-1 protease. Moreover, the central domain of p6* is unlikely to modulate protease activity (515).

Protease-Vif Interaction

Findings from coimmunoprecipitation assays, ELISAs, and HIV-1 binding assays suggest that HIV-1 Vif physically interacts with protease (282, 516, 517). To inhibit the premature activation of viral protease, HIV-1 Vif interacts with protease to interfere with protease dimerization (516–518). The binding of Vif to protease might take place inside HIV-1-infected cells prior to viral budding, potentially interrupting the autoprocessing of Gag and GagPol precursors (518). Accumulated evidence suggests that Vif selectively inhibits protease-mediated proteolytic processing at specific cleavage sites (e.g., matrix | capsid [518] and SP1 | NC [307]). Although it remains debated, the Vif-protease interaction is speculated to inhibit the protease digestion of cellular cofactors (516, 519).

Regarding the interaction domains, the N-terminal domain of protease (positions 1 to 9 [516, 517]) may interact with the central domain of Vif (positions 78 to 98 [516, 519]). Of interest, Vif-derived peptides (positions 21 to 65 [520], 30 to 65 [519], 81 to 88 [521], 78 to 98 [519], and 88 to 98 [521]) may efficiently inhibit HIV-1 protease activity.

Protease-RT Interaction

Data from coimmunoprecipitation analyses and ELISAs suggest a direct interaction between HIV-1 protease and RT (522). On the one hand, HIV-1 heterodimeric RT contributes to efficient protease-mediated proteolytic processing at many cleavage sites (e.g., RT-integrase) (523). HIV-1 RT upregulates protease activity in a concentration-dependent manner, but this upregulation is independent of pH and ionic strength (523). On the other hand, HIV-1 protease may inhibit DNA synthesis by heterodimeric RT (522). However, HIV-1 protease does not affect the activity of the RNase H domain in RT (522). Future studies are still needed to verify the protease-RT interaction, because this interaction has been reported by only a single study.

Regarding the interaction positions, alanine substitutions (T128A, Y146A, W398A, W401A, and W406A) in RT severely impair the efficiency of protease cleavages on Gag and GagPol precursors (524, 525). The decreased stability of RT mutants (e.g., F130W), however, increases the susceptibility of HIV-1 RT to protease-mediated degradation (526). Moreover, HIV-1 RT substitutions (e.g., L264S, I274T, L279S, and L310S) induce the misfolding and misprocessing of GagPol precursors, leading to the impairment of protease-mediated cleavages (527).

Protease-Nef Interaction

HIV-1 protease cleaves most Nef molecules at a location between positions W57 and L58 (AW^{57} | $L^{58}E$) (528–531), while the cleavage site in HIV-2 Nef is located between Y39 and S40 (EY^{39} | $S^{40}Q$) (532). This protease-mediated cleavage may take place inside HIV particles given the fact that a small amount of Nef is incorporated into HIV virions (286, 531, 533). Being independent of Nef myristoylation (532) and CD4 downregulation (534), this cleavage dissociates Nef into two parts: the N-terminal myristoylated membrane anchor domain and the C-terminal core domain (286, 530, 533). The former domain determines Nef packaging and CD4 downregulation (535). The latter domain might be stably associated with the viral core within mature HIV-1 particles (536). Full-length Nef inhibits protease activity, while the absence of Nef could decrease the production of mature viral particles (537). In comparisons of the activities of HIV-1 and HIV-2 proteases, it was found that Nef is cleaved more selectively by HIV-1 protease than by HIV-2 protease (532).

Regarding the cleavage sites, the fully conserved residue W57 and the relatively conserved residue L58 in HIV-1 Nef have been demonstrated in large-scale sequence data sets (4). Mutagenesis analyses also suggest that the alanine substitution W57A substantially decreases the efficiency of Nef processing, whereas L58A has little to no impact (530).

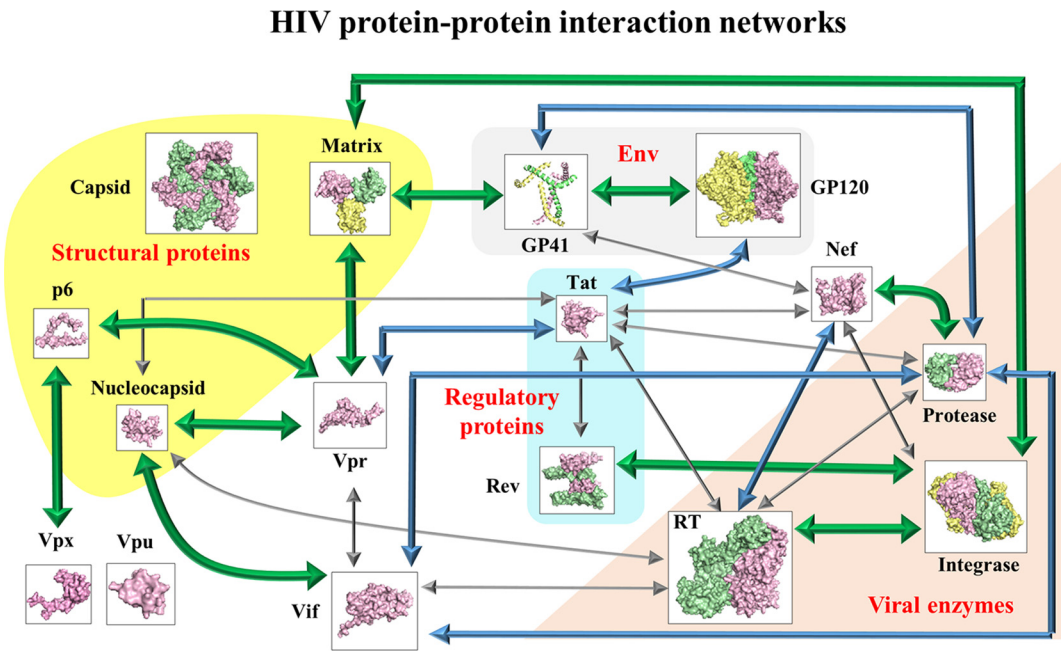


FIG 11 HIV protein-protein interaction networks. Surface or cartoon representations of 16 HIV protein structures are shown in squares. Three types of colored arrows represent HIV pairwise protein interactions between 16 mature HIV proteins. Green arrows, well-known interactions that have been cited >300 times or have been reported by at least 3 publications with more than 100 citations in total (see Table S2 in the supplemental material); gray arrows, little-known interactions that have been reported by a single paper with fewer than 100 citations; blue arrows, lesser-known interactions, including the remaining interactions. Four protein groups are shown background areas. Gray, Env glycoproteins; blue, regulatory proteins; pink, viral enzymes; yellow, structural proteins. According to our current knowledge, neither mature capsid nor Vpu physically interacts with any other HIV proteins.

Protease-GP41CT Interaction

During viral maturation, HIV-1 protease cleaves the GP41CT (538, 539). This cleavage produces large truncations in the GP41CT, causing resistance to an entry inhibitor called amphotericin B methyl ester (AME) (538, 539). Three protease-mediated cleavage sites (P^{203} | L^{204}, S^{205} | F^{206}, and R^{236} | L^{237}) at the GP41CT generate large truncations of ~140 amino acids, a mechanism that has been speculated to induce conformation changes to the external regions of Env glycoproteins (538, 539). Although the protease-mediated cleavage of the GP41CT allows the virus to develop AME resistance, large truncations of the GP41CT may impair viral entry and reduce viral fitness (539). Apart from the fact that AME has not been approved for HIV treatment, it remains unclear whether the cleavage of the GP41CT takes part in other biological activities.

ABSENCE OF HIV PAIRWISE PROTEIN INTERACTIONS

Although more than 34 HIV pairwise protein interactions have been discovered (Fig. 11 and 12), the absence of several HIV protein interactions has also been investigated.

Absence of Vif-Capsid Interaction

A possible association between Vif and capsid^{Gag} was initially proposed because short deletions in capsid^{Gag} (positions 284 to 304 and 350 to 362) may enhance Vif packaging (306). Independent analyses, however, suggested that Vif could not interact with mature capsid in HIV-1-infected H9 cells (303). In fact, Vif packaging does not require a direct interaction between Vif and capsid^{Gag}; instead, it requires the binding of Vif to NC^{Gag} and/or to viral genomic RNA (300–303, 311).

Absence of p6^{Gag}-Vif Interaction

It has been shown that p6^{Gag} is dispensable for Vif packaging, because a complete truncation of p6^{Gag} does not prevent Vif packaging (540). As a matter of fact, Vif packaging requires the binding of Vif to NC^{Gag} and/or viral genomic RNA (300–303, 311).

Absence of Matrix-Nef Interaction

HIV-1 matrix is dispensable for Nef-enhanced virus infectivity (541), even though the serine phosphorylation of HIV-1 matrix is promoted by the interaction between Nef and the human serine kinase PAK65 (542). More importantly, results from GST pulldown assays suggest that Nef cannot physically interact with HIV-1 Gag (543).

Absence of p6*-Nef Interaction

An early study suggested that Nef might interact with the transframe domain p6* in GagPol precursors (Fig. 10) by using GST pulldown and coimmunoprecipitation assays (543, 544). It was proposed that this interaction could enhance Nef packaging and viral infectivity (543, 544). However, subsequent analyses refuted these results. First, clustered substitutions in p6* did not affect Nef packaging (545). Second, viral infectivity is reduced in HIV-1 mutants with 40 substitutions of the 56 total residues in p6*, but this phenomenon is observed to be in a Nef-independent manner (545). Third, Nef packaging is mediated by the plasma membrane via a bipartite membrane location signal of Nef (533). Importantly, Nef can be packaged in the absence of GagPol (533). Fourth, the presence of Nef inside viral particles does not increase HIV-1 infectivity (546). To unveil how Nef improves viral infectivity, a recent study sug-

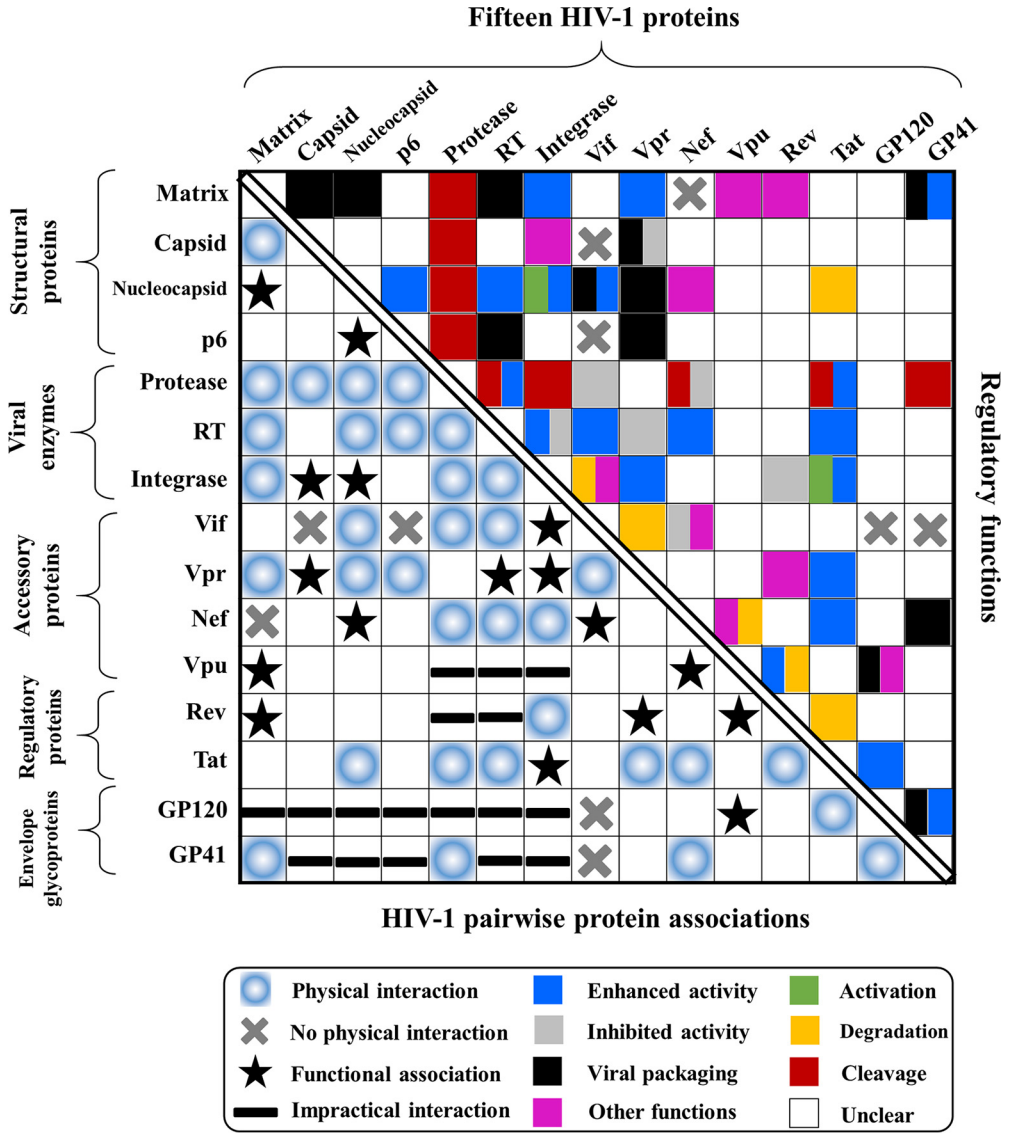


FIG 12 Summary of possible HIV-1 pairwise protein associations. The names of 15 HIV-1 proteins are shown at the left and top. Five HIV-1 protein classes (envelope glycoproteins, regulatory proteins, accessory proteins, viral enzymes, and structural proteins) are shown on the left. The biological functions of HIV-1 pairwise protein associations are shown at the top right. Protein functions are mapped with different colors. If one protein association bears multiple functions, at most two major functions are annotated, and the mapped squares combine different colors inside. At the bottom is a summary of HIV-1 pairwise protein associations. Black stars indicate protein associations where two HIV-1 proteins are associated via a third molecule (Table 3), and black horizontal lines indicate impractical interactions between HIV-1 proteins, which are unlikely to take place during the HIV-1 life cycle (see the text for details). Empty squares without any mark inside imply that HIV protein associations or interactions remain unclear. Note that interactions involved with Gag and Env precursors are decomposed into individual protein interactions. For instance, the Gag-RT interaction (347) is decomposed into the matrix-RT and p6-RT interactions. The Gag-Vpr interaction leads to the NC-Vpr and p6-Vpr interactions. Matrix^{Gag} connecting with capsid^{Gag} in Gag results in the matrix-capsid interaction. Due to the limited number of reports on HIV-2, only information on HIV-1 protein associations is presented.

gests that Nef inhibits the packaging of the host transmembrane proteins SERINC3 and SERINC5 to counteract their antiviral activity that inhibits HIV-1 production in primary human blood cells (547). Overall, p6* is unlikely to take part in Nef packaging or in Nef-mediated viral infectivity.

Absence of Vif-Env Interaction

An early study suggested that Vif may regulate Env structural conformations via its interaction with the GP41CT (548). Nevertheless, three subsequent studies refuted such an association by demonstrating that (i) the replication capacity of HIV-1 mutants with deletions in the GP41CT was independent of Vif activity (549); (ii) Vif functions are unrelated to Env, and Vif does not influence Env packaging (550); and (iii) GP41 is intact in cells infected with either the wild type or a Vif mutant (551).

Absence of Vpx-NC^{Gag} Interaction

Data from yeast two-hybrid assays suggest that SIV Vpx does not interact with NC^{Gag} (314), although HIV-1 Vpr interacts with both NC^{Gag} and p6^{Gag} (315, 316, 324).

Absence of Other HIV Protein Interactions

Based on protein activities and localizations during the HIV-1 life cycle, the absence of the following HIV-1 protein interactions could be speculated based on our current knowledge:

1. HIV-1 Vpu circulates mainly in the cytoplasm, and it is rarely observed in the nucleus, the RTC, the PIC, and nascent HIV-1 particles, where protease, RT, and integrase are localized (Fig. 3). It is therefore tempting to speculate that HIV-1 Vpu might be unlikely to interact with these viral enzymes during the HIV-1 life cycle.
2. GP120 is an envelope glycoprotein anchored mostly on cellular membranes and the membrane surface of HIV-1 particles (Fig. 5). This limits the possible interactions between GP120 and HIV structural/enzymatic proteins (matrix, capsid, nucleocapsid, p6, protease, RT, and integrase).
3. GP41 in Env spikes is anchored mostly on cellular and viral membranes. GP41 is cleaved by protease and interacts with matrix, whereas it is absent in the nucleus, the viral core, the RTC, and the PIC (Fig. 7). It is thus tempting to speculate that GP41 might be unlikely to interact with either capsid, nucleocapsid, p6, RT, or integrase.
4. Rev is absent in HIV particles, the RTC, and the PIC. Thus, Rev might be unlikely to physically interact with HIV-1 protease and RT to affect their enzymatic activities.

In addition to the above-mentioned speculations, other HIV pairwise protein interactions are yet to be discovered. Here, we present a few examples. (i) A direct interaction between Vpr and Nef has not been reported, but Vpr is required for Nef expression from the unintegrated HIV-1 DNA during preintegration transcription (318). (ii) Although it remains unclear whether $p6^{Gag}$ physically interacts with $capsid^{Gag}$, amino acid mutations in HIV-1 $p6^{Gag}$ may interrupt the proteolytic cleavage between $capsid^{Gag}$ and $SP1^{Gag}$ (552). (iii) Neutralizing responsiveness to Nef and glycosylated Gag is determined by GP120 V1/V2 regions (344), whereas it remains unclear whether GP120 physically interacts with Nef or glycosylated Gag. (iv) A recent study speculates a possible interaction between integrase and capsid (553), but experimental evidence is still lacking. Overall, future investigations of HIV genome-wide interactions are still needed to unveil all possible HIV protein interactions and to characterize their biological mechanisms and interaction domains.

CLINICAL RELEVANCE AND THERAPEUTIC IMPLICATIONS

Despite the many anti-HIV inhibitors approved by the FDA, a curative vaccine or drug against worldwide HIV infections has not been discovered (4, 554, 555). To enlighten the possible applications of HIV genome-wide protein associations, this section focuses on the clinical relevance and therapeutic implications from two aspects. First, novel mechanisms of HIV drug resistance are discussed to shed light on why some HIV-infected patients have failed antiviral treatments without drug-resistant mutations in drug-targeted proteins. Second, we provide an overview of HIV-derived peptide inhibitors that target viral enzymes on the basis of HIV pairwise protein interactions.

Novel Mechanisms of HIV Drug Resistance

Why do some HIV-infected patients fail highly active antiretroviral therapies (HAARTs) without detectable drug-resistant mutations in drug-targeted proteins? HIV genome-wide protein associations may provide insights into this mystery. It is known that drug-resistant mutations in drug-targeted proteins lead to treatment failure in HIV-infected patients (556). However, recent studies also suggest that protein-protein interactions may provide novel drug resistance mechanisms for HIV to escape the inhibition of antiviral drugs (4, 85, 557, 558). In principle, current findings rely on the hypothesis that the drug resistance of anti-HIV inhibitors could be established by sequence changes outside drug-inhibited proteins, owing to the physical interactions between drug-inhibited proteins and other HIV proteins. In fact, HIV protein interactions have a strong impact on viral genomic diversity, potentially affecting antiviral treatments and vaccine outcomes (44). Here, we provide proof-of-concept examples to highlight novel mechanisms of drug resistance driven by HIV genome-wide protein associations.

GP120 mutations may confer resistance to GP41 inhibitors. As an approved entry inhibitor, enfuvirtide (T20) is a peptide with 36 amino acids derived from the C-terminal heptad repeat region of HIV-1 GP41 (559). Sequence changes in this heptad repeat are known to confer drug resistance to enfuvirtide (556). However, the V3 loop of GP120, which interacts with host coreceptors (e.g., CCR5), also harbors mutations that confer resistance to enfuvirtide (560, 561). In an independent manner, both the GP41 heptad repeat and the GP120-coreceptor interaction contribute to sensitivity to enfuvirtide (560). To explain this finding, a mechanism has been proposed whereby conformation changes in GP41 are driven by the GP120-coreceptor interaction, thereby affecting sensitivity to the entry inhibitor enfuvirtide (561).

GP41 mutations may confer resistance to CCR5 and protease inhibitors. It is known that CCR5 inhibitors (e.g., maraviroc) interrupt virus entry by inhibiting the CCR5-GP120 interaction; therefore, sequence changes in GP120 may induce drug resistance to CCR5 inhibitors (556, 562). Surprisingly, clinical and experimental evidence suggests that GP41 mutants (e.g., G516V, M518V, and F519I) confer resistance to the CCR5 inhibitor vicriviroc (563–565). To explain this observation, a novel mechanism has been proposed whereby sequence changes in GP41 may shift the GP120-GP41 interaction to compensate for different conformations of CCR5, causing resistance to vicriviroc (566).

HIV-1 protease cleaves the GP41CT (538, 539), while the GP41CT physically interacts with the matrix region of Gag (78). Because of this physical interaction, GP41 mutations can impair protease-mediated proteolytic processing, conferring resistance to protease inhibitors (85). Although clinical evidence seems to support this mechanism, the list of GP41 mutations associated with protease drug resistance has yet to be fully described.

Integrase mutations may confer resistance to RT inhibitors. HIV integrase physically interacts with viral RT (101–106), driving integrase mutations to confer resistance to RT inhibitors (RTIs) (e.g., efavirenz) (557). For instance, viruses with integrase mutations (G140S and Q148H) and the RT mutation K103N have significantly increased fold changes in 50% inhibitory concentrations (IC_{50}s) of efavirenz compared to those of viruses with the RT drug-resistant mutation K103N alone (557). This finding is in agreement with data from viral fitness analyses, which suggest that integrase mutations (G140S and Q148H) rescue the replicative fitness of HIV-1 harboring RT drug-resistant mutations (K103N and E138K) under the selective pressure of efavirenz (557). The

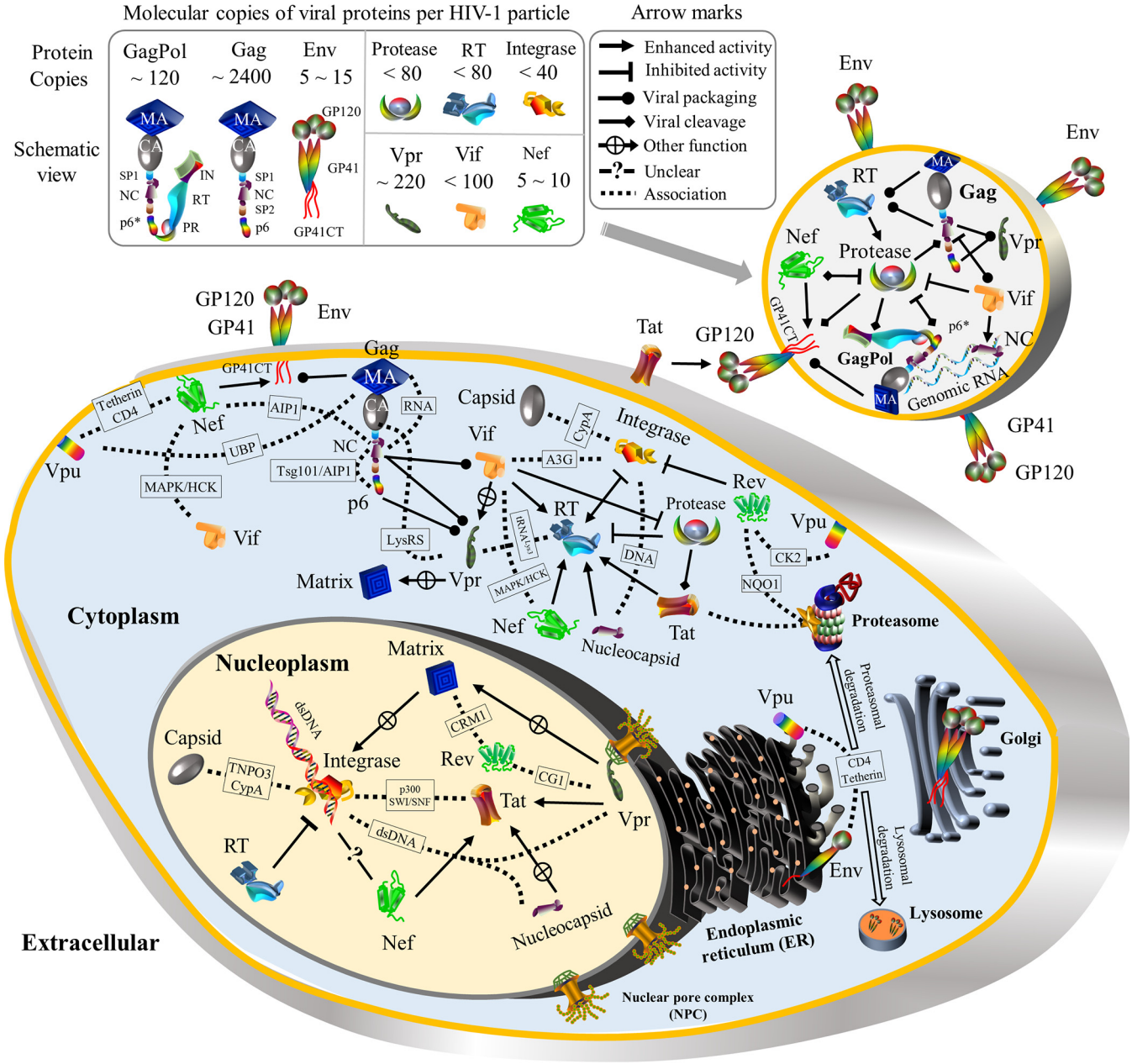


FIG 13 Overview of HIV-1 pairwise protein associations during the HIV-1 life cycle. The approximate numbers of viral proteins per HIV-1 particle are annotated in the box at the top left (see Viral Assembly and Budding). Direct physical interactions between HIV-1 proteins are shown by different arrow marks, annotated in the key at the top. If an interaction exerts multiple activities, only the major function is annotated by an arrow mark. Dash lines demonstrate functional protein associations in which two HIV-1 proteins interact with a third molecule, whose name is placed within a text box. Tables 1 and 3 summarize physical protein interactions and functional associations at each stage of the HIV-1 life cycle. Detailed signaling pathways and mechanisms of action are described in the text. Annotated protein associations indicate only their presence at a certain stage of the HIV-1 life cycle and do not indicate the simultaneous existence of all possible associations. Note that protein shapes do not represent the exact protein structures, nor are the protein sizes to scale.

list of RTI-resistant mutations outside the RT coding region has yet to be fully described.

RT mutations may confer resistance to integrase inhibitors. HIV RT physically interacts with viral integrase (101–106), permitting RT mutations to confer resistance to integrase inhibitors (e.g., raltegravir) (557). HIV-1 strains with RT mutations (E138K) and integrase mutations (G140S and Q148H) have significantly increased IC_{50}s of raltegravir compared to those of viruses with integrase mutations alone (557). This observation is in agreement with viral fitness changes in which RT drug-resistant mutations (K103N and Y181C) rescue the replicative fitness of viruses harboring integrase drug-resistant mutations under the selective pressure of raltegravir (557). In the absence of drug pressure, protease and RT drug-resistant mutations may decrease the replica-

TABLE 4 HIV-derived peptide inhibitors that interfere with HIV protein associations

HIV protein association	Drug target(s)	HIV protein(s) (positions), sequence of HIV-derived peptide inhibitor[a]	Mechanism(s) of drug action	Reference
RT-integrase	Integrase	RT (176–195), PDIVIYQYMDDLYVGSDLEI	RT-derived peptides inhibit 3′-end processing and the strand transfer reaction of integrase	575
	Integrase	RT (366–385), KQLTEAVQKITTESIVIWGK		
	Integrase	RT (396–415), ETWETWWTEYWQATWIPEWE		
	RT	Integrase (46–65), KGEAMHGQVDCSPGIWQLDC	IN-derived peptide binds to RT and inhibits RT activity	576
Vpr-integrase	Integrase, RT	Vpr (57–71), VEAIIRILQQLLFIH	Vpr-derived peptides inhibit activities of integrase and RT	162
Vpr-RT	Integrase, RT	Vpr (61–75), IRILQQLLFIHFRIG		
Integrase-Rev	Integrase	Rev (13–23), FRKLIYLTKVL	Rev-derived peptides bind to integrase and inhibit enzymatic activities of HIV-1 integrase	174
	Integrase	Rev (53–67), GLYRTSPSGRIWSI		
	Rev	Integrase (66–80), WTHLEGKIILVAVHVA	Integrase-derived peptides abrogate the inhibitory effect of Rev upon viral integration	180
	Rev	Integrase (118–128), WGSNFTSTTVKA		
Protease-Vif	Protease	Vif (1–9), MENRWQVMI	The N-terminal domain of Vif inhibits the enzymatic activity of HIV-1 protease	516
	Protease	Vif (21–65), WKSLVKHHMYVSGKARGWFYRHHYESPHPRISSEVHIPLGDARLV		520
Protease-p6*	Protease	p6* (65–68), SFNF	The C terminus of p6* inhibits HIV-1 protease activity	508
	Protease	PR (1–5), Tat (49–61), p6* (65–68), PR (95–99), PQITLRKKRRQRRRPPQVSFNFATLNF	Inhibits protease dimerization and activities	580

[a] Peptide information begins with the HIV protein name followed by the peptide-derived region in the HIV protein and the peptide sequence. For instance, "RT (176–195), PDIVIYQYMDDLYVGSDLEI" shows a peptide with the sequence "PDIVIYQYMDDLYVGSDLEI," which is derived from HIV RT between amino acid positions 176 and 195. Only representative peptide inhibitors with potent inhibitory activity were collected from the literature. Note that p6* is a transframe region in GagPol precursor proteins (Fig. 10).

tive fitness of viruses harboring integrase mutations (567). A list of INI-resistant mutations outside the integrase coding region has yet to be fully described.

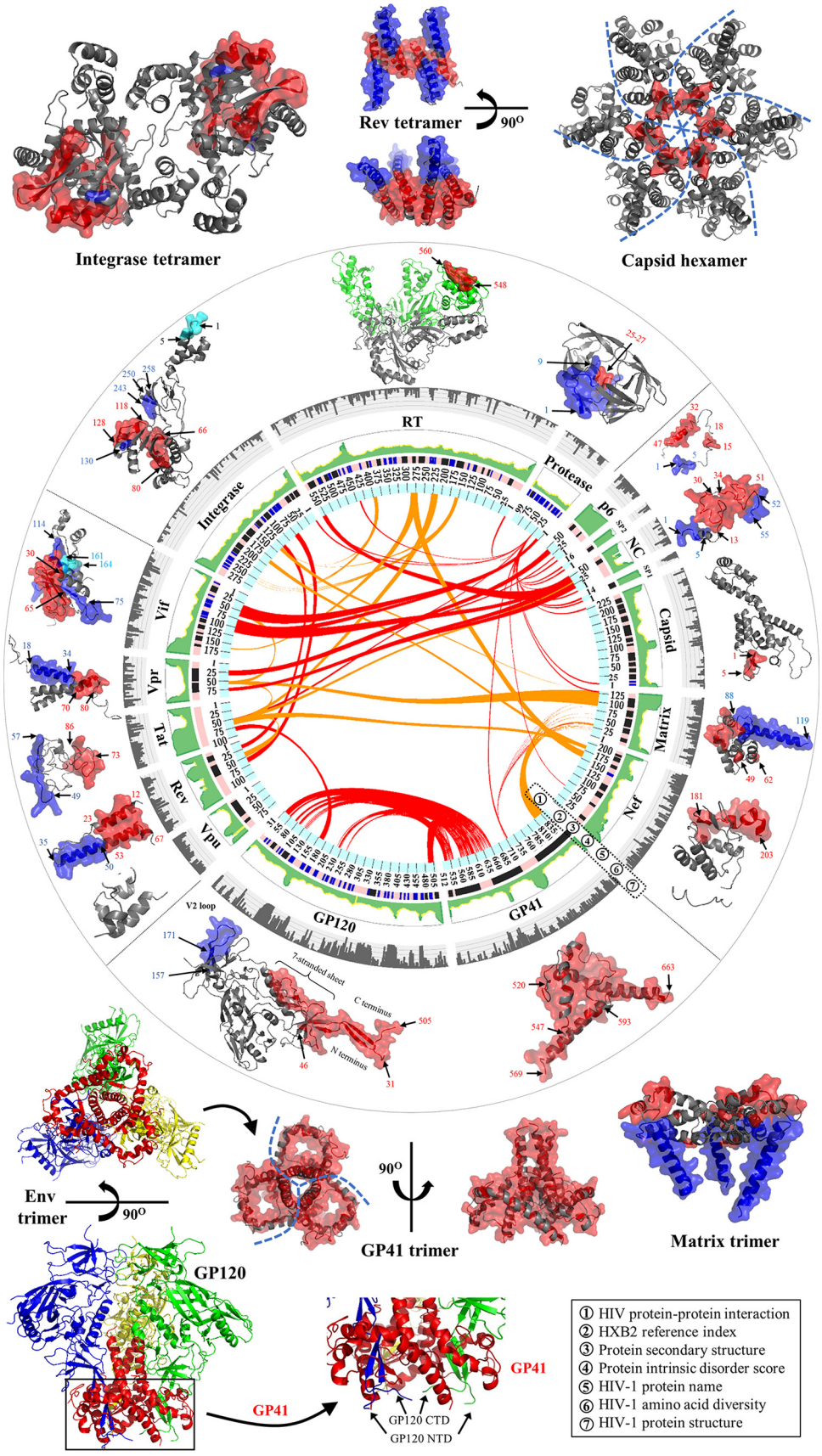
Gag mutations may confer resistance to protease inhibitors. HIV protease cleaves Gag precursors during viral maturation (Fig. 10). Amino acid mutations in Gag may cause an impaired enzymatic activity of HIV protease, inducing treatment failure of PI-based therapies in patient populations (502, 504, 505). Clinical and experimental studies have generally agreed that the protease-mediated processing of Gag establishes an alternative mechanism for HIV to select specific amino acid mutations in Gag, thus conferring resistance to protease inhibitors (502, 504, 505). In analyses of Gag amino acid mutations associated with PI resistance, it has been shown that PI-associated Gag mutations are located mainly at the C-terminal domain and cleavage sites of Gag (502, 504, 556, 568, 569). Moreover, Gag mutations such as V128I, Y132F, K415R, Q430R, A431V, L449FV, S451GT, R452S, and P453TL are significantly associated with protease drug resistance (502, 505). As new PI-associated Gag mutations have been consistently reported (558, 570), further studies are required to map all PI-associated Gag mutations and to evaluate their impacts in studies with large patient populations.

Vif mutations may confer resistance to protease inhibitors. HIV-1 Vif can physically interact with protease during viral maturation (282, 516, 517). In analyses of Vif sequences sampled from PI-treated and PI-naive patients, amino acid substitutions at five positions of Vif (R36, P47, E101, D117, and L124) are associated with protease drug resistance (571). Due to the limited number of copies of Vif molecules incorporated into HIV particles (Fig. 13), a question that remains to be explored is whether Vif-associated drug resistance plays a significant role in large patient populations.

Apart from the novel mechanisms of HIV drug resistance described above, other HIV pairwise protein associations may have provided alternative pathways for HIV to develop resistance against anti-HIV drugs, marking a new era for the investigation of HIV drug resistance. Importantly, our survey indicates that HIV genomic sequencing is needed to detect novel drug-resistant mutations occurring outside drug-targeted regions, whereas this strategy has not been implemented in current commercial genotypic or phenotypic resistance assays. Overall, a comprehensive survey of HIV genome-wide protein associations sheds light on novel mechanisms of HIV drug resistance from a genome-wide perspective, contributing to the advancement of antiviral treatments.

Development of HIV-Derived Peptide Inhibitors

Based on publications accumulated over the past 3 decades, our review provides a comprehensive overview of HIV pairwise protein associations at different stages of the HIV life cycle, potentially contributing to the development of anti-HIV inhibitors. In light of the FDA approval of enfuvirtide (572, 573), this section



highlights HIV-derived peptide inhibitors being developed based on HIV pairwise protein associations (Table 4).

RT-integrase interaction. On the one hand, peptide inhibitors derived from HIV-1 RT (positions 166 to 185 and 519 to 532) can efficiently inhibit 3′-end processing and the strand transfer reaction of integrase (574, 575). On the other hand, a peptide derived from HIV-1 integrase (positions 46 to 65) can inhibit the polymerase activity of HIV-1 RT (576) (Table 4).

Vpr-integrase association. Peptide inhibitors (positions 58 to 72, 57 to 71, and 61 to 75) derived from an alpha-helix structure of Vpr can inhibit the activity of HIV-1 integrase (162, 577). By screening a peptide library built from HIV-1 protein sequences, Vpr-derived peptides containing the motif L^{64}QQLLF^{69} exhibit promising inhibitory activity against HIV-1 integrase (578).

Vpr-RT association. Although RT-binding domains in Vpr remain unclear, peptides derived from two Vpr regions (positions 57 to 71 and 61 to 75) physically interact with HIV-1 RT to inhibit reverse transcription (162).

Integrase-Rev association. Rev-derived peptides (positions 13 to 23 and 53 to 67) efficiently inhibit the enzymatic activity of integrase (180). Moreover, integrase-derived peptides (positions 66 to 80 and 118 to 128) can rescue Rev-mediated inhibitory effects (180).

Protease-Vif association. Peptide inhibitors derived from the N-terminal domain of HIV-1 protease (positions 1 to 9) can mimic the interface of the protease-Vif interaction to block protease-mediated proteolytic processing (517). Moreover, Vif-derived peptides (positions 30 to 65 [519], 41 to 65 [520], 78 to 98 [519], 81 to 88 [521], and 88 to 98 [521]) efficiently inhibit protease activity.

Protease-p6* interaction. A peptide inhibitor derived from four parts (p6* CTD, Tat cell-permeable domain, and protease NTD and CTD) (Table 4) blocks protease dimerization and interrupts protease-mediated proteolytic processing (579, 580). Note that four amino acids (S^{65}FNF^{68}) at the p6* CTD are essential for inhibiting the activity of viral protease (508).

Overall, anti-HIV peptides derived from protease, RT, integrase, Vif, Rev, Vpr, and p6* have shown potent activities against enzymatic activities of HIV protease, RT, and integrase. For this reason, an in-depth understanding of HIV genome-wide protein interactions may provide insights into anti-HIV drug development.

CONCLUSIONS AND FUTURE PERSPECTIVES

Armed with publications accumulated over the past 3 decades, this review provides for the first time a comprehensive overview of HIV genome-wide protein associations at major stages of the HIV life cycle (Fig. 13). Our genome-wide perspective on HIV pairwise protein associations reveals intrinsic cross talk between HIV proteins, contributing to the investigation of novel drug resistance and the development of novel antiviral agents (581–584). Bear in mind that the HIV genome encodes only 16 proteins; a high level of HIV pairwise protein associations is therefore expected. Given 120 possible HIV pairwise protein associations between 16 viral proteins, our review summarizes experimental evidence on 34 direct physical interactions (Fig. 11) and 17 functional associations (Fig. 12). To provide a global perspective of HIV genome-wide protein interactions, we have also mapped the protein interaction domains to HIV protein structures, along with integrated information on protein secondary structure, protein intrinsic disorder, and protein sequence diversity (Fig. 14). Overall, this in-depth overview of HIV genome-wide protein associations reveals a high level of mutual collaborations between HIV proteins during the HIV life cycle.

Our survey suggests that every HIV protein is associated with another viral protein. Among 16 viral proteins, only HIV-1 Vpu and structural proteins are unlikely to interact with other viral proteins due to their functional roles (see Text S1 in the supplemental material). HIV-1 Vpu is known to interact with host proteins for CD4 downregulation and tetherin antagonism (585), while structural proteins (e.g., capsid) are expected to maintain stable HIV structures with lesser associations (503). In comparison, HIV-1 regulatory and accessory proteins (Tat, Rev, Vpr, Vif, and Nef) have more opportunities to engage in a dialogue with other viral and host proteins in many cell compartments, because they undertake multiple activities during the HIV life cycle (Fig. 13).

Despite many findings accumulated over the past 3 decades, investigation of HIV pairwise protein associations from a genome-wide perspective is still warranted. Previous studies unveiled the global landscape of HIV-host protein interactions from high-throughput data (6–11). However, most of these studies have underestimated HIV pairwise protein interactions, providing little to no information on the sophisticated associations between HIV proteins. Exploration of HIV genome-wide protein associations requires the accurate detection of protein associations at different stages of the HIV life cycle. Here, we highlight several challenges for future studies.

1. HIV macromolecular structures such as the RTC and the PIC have not been resolved in spite of countless attempts being made over the past decades.
2. A single protein interaction (e.g., RT-integrase) may have different activities at several HIV life stages, making the elucidation of such interactions difficult.
3. HIV proteins harbor intrinsically disordered structures to interact with other proteins (44, 586). Intrinsically disor-

FIG 14 Integrated map of HIV-1 pairwise protein interactions in the full-length genome. Fifteen HIV-1 proteins are plotted in a circle with seven layers. In layer 1, red links in the center indicate interaction domains for HIV-1 pairwise protein interactions (Table 1). Orange links indicate physical interactions between HIV-1 proteins, but their interaction domains are yet to be resolved (Table 1). In layer 2, indices of amino acid positions are annotated by using HIV-1 HXB2 as a reference. Layer 3 shows protein secondary structures (dark blue, helix structures; light blue, beta-strand structures; pink, random-coil structures) (44). In layer 4, intrinsic disorder scores of individual amino acid positions are shown in green. The range of intrinsic disorder scores is between 0 and 1 (the higher the value, the higher the structural variability) (44). In layer 5, 15 HIV-1 proteins have their names annotated accordingly. In layer 6, amino acid genetic diversity of the HIV-1 subtype B genome is exhibited in gray. Diversity values of between 0 and 1 are mapped on five sublayers (44). In layer 7, protein interaction domains are mapped on cartoon representations of crystallized HIV-1 protein structures. HIV-1 multimeric proteins are shown outside the circle. Data sets for HIV-1 intrinsic disorder scores and amino acid diversity were gathered from our recent report (44). Human proteins involved in HIV-1 pairwise protein associations are not illustrated. See Table S1 in the supplemental material for a list of PDB accession numbers used for structural visualization. PyMOL V1.7 (see http://www.pymol.org/) and Circos V0.64 (http://circos.ca/) visualization software were used.

dered structures usually have dynamic forms, which might hinder accurate detections of protein interaction domains (586).

4. Many HIV pairwise protein interactions and their accurate interaction domains have yet to be fully described (Fig. 14 and Table 1). Of 34 pairwise protein interactions, more than 10 interactions are known, and their biological functions are well characterized in the literature (Fig. 11). Nevertheless, future studies are still required to address the reproducibility of the other HIV protein interactions, especially those interactions reported in only a single article (Table 2).
5. Many experiments performed in cell-free settings or with nonnatural target cells may have underestimated the nature of HIV protein interactions because of different protein expression levels and/or the lack of host proteins in cell-free and nonnatural settings. Furthermore, cell type-specific factors in different HIV strains might have been underestimated, because most cell culture experiments have been performed by using cell lines infected with HIV-1 subtype B strains (e.g., HXB2). Therefore, the development of cell culture experiments that represent dynamic protein interactions in a real biological context remains a challenge.

Our review focuses mainly on HIV-1 and less on HIV-2/SIV, because HIV-1 causes major infections worldwide, and the number of reports on HIV-2 and SIV is limited. Although both HIV-1 and HIV-2 originated from SIV (587), they have distinct gene maps (Fig. 1). Particularly, Vpu in HIV-1 and Vpx in HIV-2/SIV, which play different roles during the HIV life cycle, mark a distinct difference (588, 589). For instance, the packaging of SIV Vpx is absolutely dependent on the $L^{41}XXLF^{45}$ motif of SIV $p6^{Gag}$ (320), but HIV-1 Vpu is not packaged into virions (590). Therefore, it warrants further investigations to explore the differences of genome-wide protein associations between HIV-1 and other simian immunodeficiency viruses (e.g., HIV-2 and SIV). On the other hand, our review identified HIV protein interactions based on keyword searches of reports published between 1985 and 2015, but additional HIV protein interactions will be (or might have been) reported in the literature. For this reason, we have established an online platform (http://www.virusface.com/) to update the information on HIV protein interactions.

Overall, our review provides insights into HIV pairwise protein associations from a genome-wide perspective, shedding light on potential therapeutic targets for drug discovery. Importantly, a comprehensive map of HIV genome-wide protein associations has been established to support the hypothesis that all HIV proteins collaborate meticulously to facilitate viral infections during the viral life cycle.

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Guangdi Li, Ph.D., received his doctoral degree in Biomedical Sciences from the Faculty of Medicine, KU Leuven, Belgium, in 2014. During his Ph.D., he received extensive training from the Master of Molecular and Cellular Biophysics, Faculty of Science, KU Leuven. In 2006, he obtained his bachelor's degree in Applied Mathematics at Hunan University. Between 2006 and 2009, he pursued his master's degree in Computer Science at Shandong University and received full scholarships to work as a research assistant at the Technical University of Madrid, Spain. Between 2009 and 2014, he undertook his doctorate training in the Faculty of Medicine, KU Leuven, Belgium. Since 2015, he has become a research fellow at the Second Xiangya Hospital, Central South University, Hunan, China. His research interests are focused on the genome-wide diversity, coevolution, and interaction of HIV, HBV, and HCV (see http://www.virusface.com/).

Erik De Clercq, M.D., Ph.D., is a professor emeritus at KU Leuven, Belgium, and a visiting professor at the University of South Bohemia in the Czech Republic. His research interests are embraced within the broad areas of microbiology, virology, molecular biology, and antiviral research, focusing on HIV, hepatitis B virus, hepatitis C virus, influenza virus, varicella-zoster virus, herpesvirus, and emerging viruses (e.g., Ebola, dengue, and chikungunya viruses). Over a period of five decades, he has developed new antiviral medicines, including nucleotide analogues (e.g., tenofovir), and he has coinvented several approved antiviral drugs, such as BVDU (brivudine), amino acyl esters of acyclovir (e.g., valacyclovir), acyclic nucleoside phosphonates (ANPs) (cidofovir, adefovir, and tenofovir), non-nucleoside reverse transcriptase inhibitors, and HEPT and TIBO derivatives leading to rilpivirine for the treatment of AIDS. Since the start of his academic career in 1967, Prof. De Clercq has published more than 2,700 research articles (see http://www.virusface.com/).